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7809 C*

A Comparison of the Resistance of Bacteria and Embryonic Tissue to Germicidal Substances. I. Merthiolate.

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Lambert[1, 2] bathed fragments of human tissues in a dilute broth culture of *Staphylococcus aureus* for a few minutes and then in dilutions of the germicides for one hour. The tissue fragments were then washed in physiological salt solution, embedded in plasma and examined for growth of bacteria and tissue after several days of incubation. The chemicals included alcohol, iodine, argyrol, mercuric chloride, potassium mercuric iodide, potassium cyanide, hypochlorites, phenol, tri-cresol and hydrogen peroxide. He found that human connective tissue cells and histiocytes, were, in general, more easily killed than *Staphylococcus aureus*. Of the compounds tested iodine approached most closely the theoretically perfect disinfectant, killing bacteria in strengths that did not seriously injure human tissue cells.

* C represents a complete, P a preliminary manuscript.

1 Lambert, R. A., *J. Exp. Med.*, 1916, **24**, 683.

2 Lambert, R. A., *J. Am. Med. Assn.*, 1916, **67**, 1300.

In a later communication Lambert and Meyer[3] modified the above procedure. Rabbit spleen was used instead of human tissue. Also, the tissue fragments were bathed in a culture of *Staphylococcus aureus* for one minute followed by 20-minute exposures to the dilutions of the germicides, before being embedded in plasma. The chemicals tested included alcohol, iodine, mercuric chloride, mercurochrome, acriflavine, protargol, albargin, gentian violet, neosalvarsan and hexylresorcinol. Iodine was again found to approach nearer the theoretically perfect disinfectant. It is possible that in the favorable cultures the cells may have come from the center of the explant where they were partially protected. In another series the chemicals were added directly to the culture medium. Lambert and Meyer arrived at the same conclusions as in the first series.

German[4] bathed fragments of chick tissues in broth cultures of *Streptococcus hemolyticus, Staphylococcus aureus* and *Bacillus coli,* after which they were transferred to the antiseptic solutions. After periods of one and 5 minutes they were planted on agar plates. Fragments of chick tissues were also bathed in various dilutions of the chemicals for one and 5 minutes, after which they were washed in Locke's solution and embedded in plasma. He believed that the efficiency of an antiseptic to be inversely proportional to its harmful action on tissues and directly proportional to its bacteriostatic effect

Buchsbaum and Bloom[5] prepared chick tissue cultures in which the various dilutions of the antiseptics were embedded in chick plasma. The test organism, *Staphylococcus aureus* was added to the overlying embryonic fluid. The cultures were observed for bacterial and tissue growth after 24 and 48 hours' incubation. They tested phenol, iodine, mercurochrome, metaphen and merthiolate. They stated that an antiseptic killing the bacteria at concentrations that would not harm the cells would have an index of 1.0 or greater (greatest dilution that killed the organisms divided by the greatest concentration in which cells show approximately normal growth). Merthiolate was given an index of 0.9, the highest and phenol 0.2, the lowest.

Our work was devoted entirely to a study of phenol and merthiolate. The test organisms were *E. typhi* and *Staphylococcus aureus.* The organisms were at no time in contact with the tissues. Phenol

[3] Lambert, R. A., and Meyer, J. R., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 429.

[4] German, W. J., *Arch. Surg.*, 1929, **18**, 1920.

[5] Buchsbaum, R., and Bloom, W., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1060.

coefficients for merthiolate were first determined, using the method of Reddish (as recommended by the A. P. H. A.).

The *E. typhi* phenol coefficient was found to be 50 and that for *Staph. aureus* 70. Identical results have been reported by Eli Lilly and Company, manufacturers of merthiolate.

Chick hearts obtained from 9-day-old embryos furnished the tissue. The tissue fragments were embedded in guinea pig plasma diluted with 3 parts of Tyrode solution and one part of embryonic fluid. After coagulation of the plasma the flasks were washed with Tyrode solution to remove the uncoagulated constituents of the plasma.

Dilutions of phenol and merthiolate were made in chick embryonic fluid and then added to the flasks. All fluids were accurately measured so that the final concentrations of the chemicals could be determined. Cultures were examined after 48 hours of incubation.

The results are given in Table I.

TABLE I.

Germicide	Highest Dilution Showing no Tissue Growth=A	Highest Dilution Showing no Growth of *E. typhi*=B	Highest Dilution Showing no Growth of *S. aureus*=B	Toxicity Index=A/B
Phenol	1-840	1-80		10.5
"	1-840		1-70	12.0
Merthiolate	1-176,400	1-4,000		44.1
"	1-176,400		1-5,000	35.3

Theoretically the smaller the toxicity index, the more efficient the germicide. In our tissue culture experiments phenol showed a smaller toxicity index than merthiolate.

The above results are just the reverse of those reported by Buchsbaum and Bloom. It is believed that the introduction of the organisms in the tissue cultures with the dilutions of the germicides by the above workers was responsible for the differences in results. Since the chemical was embedded in the plasma and the organisms were suspended in the overlying embryonic fluid it is very questionable if a uniform mixture of germicide and organisms was obtained. Also chick rather than human plasma was used. Chick plasma may exert a bactericidal action on the organisms which may be wholly lacking, or almost so, in human plasma.

7810 C

Portal and Hepatic Blood Sugar After Glucose Administration.

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Olmsted and Read[1] found that in the decapitate cat there was more glucose in hepatic than in portal blood at a time when glycogen was being deposited in the liver. At the same time Tsai and Yi[2] obtained similar results, and also reported[3] that even during absorption of sugar after its injection into the stomach, the concentration of sugar in portal blood in the decapitate cat falls short of that in hepatic blood. We had at the same time completed a similar investigation of the decapitate cat, and had extended our experiments to the amytalized dog, taking into account the behavior of lactic acid in the blood. The data are as follows.

We injected 0.5 gm. glucose per kilo body weight into the duodenum of 2 decapitate cats, the hepatic artery having been ligated in each. Total reducing substance was estimated by Folin-Wu tungstate precipitation; glucose by Somogyi's zinc precipitation; 0.1 cc. samples were used.

In these 2 preparations, blood taken from the portal vein within half an hour after glucose injection contained more glucose than hepatic blood. But within an hour the situation was reversed (Table I).

TABLE I.

Total reducing substance and glucose in hepatic and portal blood of decapitate cat with hepatic artery tied.

6.5 cc. of 40% glucose sol. injected 4 hr. after decapitation.	Hepatic		Portal	
	"Blood Sugar"	Glucose	"Blood Sugar"	Glucose
20 min. after glucose	216	190	247	229
1 hr. " "	260	237	240	220
2 hr. " "	325	300	306	290
4 hr. " "	211	186	200	178

In a third preparation, in which the hepatic artery was not tied, there was delay in absorption and more glucose was found in hepatic than in portal blood in every case (Table II).

[1] Olmsted, J. M. D., and Read, L. S., *Am. J. Physiol.*, 1934, **109**, 303.

[2] Tsai, C., and Yi, C. L., *Chinese J. Physiol.*, 1934, **8**, 245.

[3] Tsai, C., and Yi, C. L., *Chinese J. Physiol.*, 1934, **8**, 273.

TABLE II.
Total reducing substance and glucose in hepatic and portal blood of decapitate cat, hepatic artery not tied.

	Hepatic		Portal	
	"Blood Sugar"	Glucose	"Blood Sugar"	Glucose
4 hr. after decapitation	120	103	99	90
½ hr. after glucose	122	114	99	90
1 hr. " "	157	147	151	140
1½ hr. " "	215	195	169	158

Three dogs under amytal anesthesia were given a solution containing 10 gm. glucose by subcutaneous injection, the hepatic artery being tied. Total blood sugar was determined by the Folin-Wu method, using 1 cc. samples. Lactic acid was determined by the Friedemann, Cotonio, and Shaffer method. Rose, Giragossintz, and Kirstein[4] found that although fructose caused a great increase in blood lactic acid, glucose brought about little, if any, change in lactic acid in portal blood. We find a slight but significant increase in lactic acid after glucose injection. The results are averaged in Table III. It will be noted that the sugar plus one-half the lactic acid (since we may consider 2 molecules of lactic acid equivalent to one of glucose) in hepatic and portal blood do not balance, *i. e.*, the extra amount of sugar in hepatic blood is not balanced by an equivalent amount of lactic acid in portal blood, although there is more lactic acid in portal than in hepatic blood.

In 7 amytalized dogs 10 gm. of glucose were injected into the

TABLE III.
Average hepatic and portal "blood sugar", lactic acid, and liver glycogen in 3 dogs after subcutaneous injection of 10 gm. of glucose.

Hr. after Glucose	Hepatic		Portal		Liver Glycogen
	Sugar	Lactic Acid	Sugar	Lactic Acid	
1	170	27	130	35	1.15
2	207	24	152	42	1.31
3	127	20	101	37	1.45

TABLE IV.
Glucose and lactic acid in hepatic, portal, and femoral artery blood of amytalized dog.

	Hepatic		Portal		Femoral Artery	
	Glucose	Lactic Acid	Glucose	Lactic Acid	Glucose	Lactic Acid
Before glucose	90	20	76	27	80	25
1 hr. after glucose	141	20	130	32	135	27
2 hr. " "	135	28	120	39	120	32

[4] Rose, M. I., Giragossintz, G., and Kirstein, E. L., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 523.

duodenum. The hepatic artery was not tied. The results of a typical experiment are given in Table IV.

TABLE V.
Average hepatic and portal "blood sugar" and lactic acid in 7 amytalized dogs.

Hr. after Glucose	Hepatic		Portal	
	"Blood Sugar"	Lactic Acid	"Blood Sugar"	Lactic Acid
1	152	22	120	30
2	240	34	180	51
5	122	18	90	26

We find, therefore, that shortly after injection of moderate amounts of glucose there may or may not be more glucose in portal than in hepatic blood; after an hour, however, while absorption is still proceeding, there is more glucose in hepatic than in portal blood, and at the same time there is more lactic acid in portal than in hepatic blood, although not enough to account for the differences in the sugar content of blood entering and leaving the liver.

7811 P

Effect of Intravenous Acacia on Physio-Chemical Properties of the Blood.

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(Introduced by C. D. Leake.)

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Stimulated by the reports of Hartmann[1] on the intravenous use of acacia, we were encouraged to give a clinical trial to certain cases of intractable nephrotic edema. This was followed by such marked diuresis and unusual reactions that it was deemed advisable to accumulate further knowledge on the subject. Extensive observations of these phenomena have been recorded by Bayliss,[2] Henderson,[3] and Hanzlik[4] but with few observations on the oxygen content of the blood.

This preliminary report of the effects of intravenous acacia solu-

[1] Hartmann, A. F., Senn, M. J. E., Nelson, M. V., and Perley, A. M., *J. Am. Med. Assn.*, 1933, **100**, 251

[2] Bayliss, W. M., *J. Am. Med. Assn.*, 1922, **78**, 1885.

[3] Henderson, Y., and Haggard, H. W., *J. Am. Med. Assn.*, 1922, **78**, 697.

[4] Hanzlik, P. J., De Eds, F., and Tainter, M. L., *Arch. Int. Med.*, 1925, **36**, 447.

tion on gaseous exchange and incidental findings, is to be followed by other observations on the physio-chemical factors of the blood.

Dogs were used as the experimental animals. The standard apparatus of Van Slyke[5] was employed for the gasometric content determinations by manometric method. Ten experiments were completed, which were uniformly consistent. A typical result is indicated in Table I.

TABLE I.
Experiment No. 7, Weight of Dog 11.8 kg., Acacia 15% in Distilled Water Given Intravenously 2.75 gm. per kg. of Body Weight.

Determinations	Initial	½ hr.	1 hr.	2 hr.	4 hr.	24 hr.	1 week
Hemoglobin	115%	97%	92%	107%	107%	105%	107%
Red Blood Cells	8.04	7.32	7.34	7.64	7.09	6.76	6.3 M
Hematocrit	44.0	41.5	39.0	45.0	45.0	42.0	41.0
Sedimentation Rate	24 hr.+	12 min.	14 min.	25 min.	32 min.	21 min.	3 hr.+
CO_2	39.16	33.46	35.01	34.24	33.11	39.43	39.11
O	14.19	8.66	9.38	8.66	9.90	9.86	10.13

It will be noted that the hemoglobin fell with the red blood cells in direct proportion to the decrease in hematocrit value or increase in blood volume, which uniformly follows the use of intravenous acacia. Rapid sedimentation of the red blood cells following intravenous acacia was early noted and stimulated the report of Lucia and Brown[6] in a recent issue of this journal.

Although the carbon dioxide content of the blood decreased following the use of intravenous acacia, proportionate to the blood dilution observed by Hanzlik,[4] the oxygen content fell at the end

TABLE II.
Experiment No. 10, Weight of Dog 9.6 kg., Acacia 15% Solution in Distilled Water Given Intravenously, 4.5 gm. per kg.

	Determinations. Initial	1 hr.	4 hr.	24 hr.
Hemoglobin	87%	70%	52%	50%
Hematocrit	34.0	27.5	21.0	20.5
Arterial Blood				
CO_2	36.01	33.89	34.42	35.82
O	20.99	13.12	9.76	10.94
Venous Blood				
CO_2	41.83	35.75	36.01	38.66
O	11.96	12.21	9.28	9.28
Venous Blood Saturated with Air				
CO_2	35.48	37.28	32.39	
O	17.82	12.79	10.94	

[5] Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry Methods. The Williams and Wilkins Co., Baltimore, 1932.

[6] Lucia, S. P., and Brown, J. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**,

of one hour to a more marked degree and persisted over a 24-hour period.

We had already noted the effect described by Walker,[7] which makes N/10 HCl necessary to dissolve the red blood cells in doing routine white blood counts following the infusion of acacia and we assumed that the acacia coated the cells and hindered normal blood cell respiration. Since fragility tests were constantly normal, we decided to attempt to resaturate the red blood cells by exposure to air. The results of this are shown in Table II.

It will be noted that it was possible to resaturate the red blood cells to a point which would preclude any possibility that the low oxygen saturation of the venous blood following intravenous acacia was not due to coating of the red blood cells.

7812

Nutritional Factors of S. Lactis.

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Whenever one is dealing with 3 variables, the trilinear chart is often found to be useful. Such a chart is based upon the thesis that in an equilateral triangle the sum of the perpendiculars from any point to the sides is equal to the altitude of the triangle. If this altitude is designated as 100%, then the position of the point within can be expressed in terms of per cent since $x + y + z = 100$.*

In our studies with *S. lactis* we have used 1% Difco peptone, 1% Difco yeast extract and water as variables. These solutions are prepared and adjusted electrometrically to pH 6.5. Media 1, 21, and 16 (see Fig.) are these 3 solutions. Each of these media contained 0.5% lactose. By properly mixing these solutions, 18 other media are made which are represented in the figure by the various numbers. Each medium varies from the other in some small degree. Thus the media in the base line (16 to 21) have no peptone. This is the zero line for peptone.

If the altitude of the triangle is now divided into 5 equal parts and lines drawn parallel to the base through these points a series of

[7] Walker, M. A., *Am. J. Clin. Path.*, 1932, **2**, 347.

* For further discussion of the theory see Haskell: How to Make and Use Graphic Charts. Codex Book Company. Page 30.

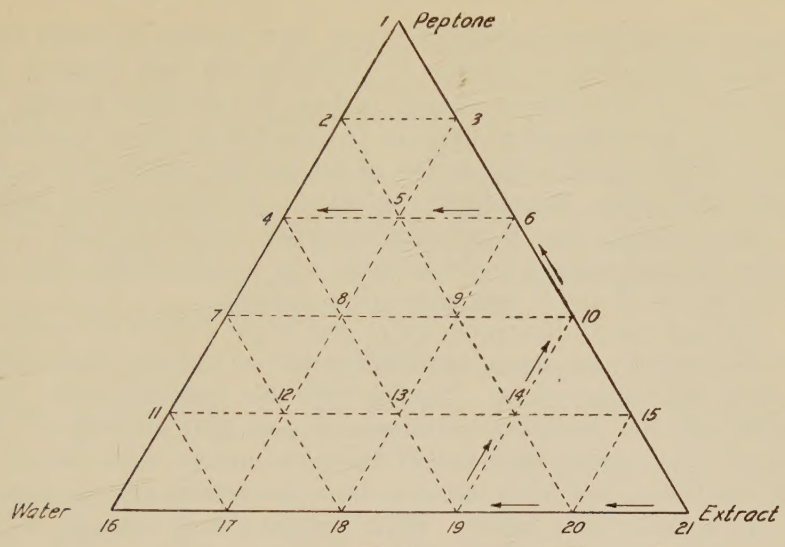

FIG. 1.
Showing the zones of similar growth in media with varying constitutions.

triangles is obtained. The media on line 11-15 will all have 0.2% peptone (20% of the original 1% solution). Also, all media on line 7-10 will have 0.4% peptone. Medium 1, of course, has 1% peptone (100% of the 1% solution). The same reasoning can be used for the extract. In the case of the water, it is merely a diluent of the media in which it appears.

With this method, some interesting results have been obtained with *S. lactis.*

These 21 media were each inoculated with 0.1 cc. of a 10^{-4} dilution of an 18-hour-old milk culture of *S. lactis.* Since the media has been distributed in tubes in 10 cc. amounts, the final dilution of the culture in the tubes was 10^{-6} These tubes had been sterilized in the autoclave and rechecked as to reaction. Little or no change was noted. After 24 hours' incubation at 30°C., the growth was recorded as amount of turbidity using ++++, +++ to designate the degree of turbidity.

Media 21 to 19 showed ++++ growth, but medium 18 gave only ++ growth. These vary only by dilution and evidently the concentration of something present in 19 is lessened in 18 to a considerable degree. However, following the "iso extract" line to medium 10 (see arrows), a ++++ growth is observed.

Medium 6, too, has +++ growth. Something must be supplied by the peptone which is not supplied by the water. Following the "iso peptone" line to medium 4 shows no growth in this latter. If

peptone supplied the necessary nitrogen, then medium 4 should support growth as well as medium 6. This has not been found to be the case. It occurred to us that perhaps the excellent growth in medium 6 as compared to medium 18 (the extract being constant) might be due to the buffer action of the peptone rather than to its nutritional value. Accordingly we made a Sörensen citrate buffer of pH 6.5 diluted 1 to 10 and substituted this for the peptone. This buffer behaved exactly as the peptone. In fact the differences between media 18 and 6 were even more marked.

Although citrate is a good buffer and might replace the peptone for this reason, it is also an excellent source of carbon. This could explain our observations. To rule out this possibility, we next made a N/100 $NaH\,CO_3$ solution and used that in place of the citrate. Our results were exactly the same and we were forced to the conclusion that for *S. lactis* at least the peptone serves largely, if not entirely, as a buffer. How general this is remains to be seen. It is obvious, too, that the possibilities in the use of this device are almost limitless.

7813 P

Effect of a Diet Low in Magnesium on the Rat.*

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Feeding a diet containing only 0.18 mg. of magnesium per 100 gm. of food, Kruse, Orent and McCollum[1] have discovered a syndrome in the rat which they term magnesium tetany. In this condition, which is characterized by a hyperexcitability manifested by spontaneous or induced convulsive seizures, these authors found the plasma magnesium content to be reduced to a low level of 1 mg. % or less.

A considerably different sequence was observed by the present writers on a diet containing between 1 and 2 mg. of magnesium per 100 gm. of dry food which was composed of casein, sucrose, vegetable fat, vitamin supplements, and a purified salt mixture. On this

* Aided by a grant from the Christine Breon Fund of the Medical School.

[1] Kruse, H. D., Orent, E. R., and McCollum, E. V., *J. Biol. Chem.*, 1932, **96**, 519; 1933, **100**, 603.

diet the animals have never been observed to suffer spontaneous convulsions, but convulsive seizures can be readily induced through application of a suitable stimulus, such as the hissing sound of an air blast. A striking observation is that the plasma magnesium content of these animals, while invariably lower than the content of the controls, falls within the range of variation which has been reported for normal animals. The body magnesium level, too, was found to be markedly reduced. / Results of the plasma magnesium and body magnesium analyses are given in Table I.

TABLE I.

Series	Time on diet wk.	No. animals in each group	Plasma Mg†		Body Mg			
			Controls	Mg deficient	Controls		Mg deficient	
			mg./100 ml.		Range mg./100 gm.	Mean	Range body weight	Mean
I	7	2	3.75	2.65	‡	42	‡	28
I	10	2	4.30	2.25	‡	32.5	‡	18
II	8	4	2.55	1.55	38-42	40	26-29	27
III	12	4	3.00	2.25	30.5-32	31	19.5-24.5	22

† The plasma magnesium values were determined on pooled blood samples.
‡ Analyses differed slightly from each other.

The period of time required to reach the condition in which the rats are subject to convulsions was found to be markedly affected by the level of the vitamin G intake. Animals fed a ration low in G reach this stage much sooner than those given larger amounts of this vitamin. With an ample quantity of vitamin G and at the level of magnesium stated, there was not developed the trophic changes such as loss of hair, emaciation and edema of the feet, which are stated by Kruse, Orent and McCollum to be characteristic of the terminal stage of the deficiency.

7814 C

Failure of Theelin and Thyroxin to Affect Plumage and Eye-Color of the Blackbird.*

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Evidence from a number of sources has made it apparent that sexual differences in the plumage of the common fowl

* Supported in part by the Rockefeller Fluid Research Fund of the Stanford University School of Medicine.

(Gallus) are largely regulated by endocrine factors. In most breeds feathers of the male type are replaced, after administration of appropriate amounts of thyroxin, theelin or crude pituitary extract, by feathers resembling those of the female. Nevertheless, it is recognized that there are pronounced differences between breeds in their response to these prepared hormones.[1] For a time it seemed possible that the mechanism of differentiation in secondary sexual characters might be essentially the same in all avian species, the variations being only quantitative in character. Evidence seeming to point in this direction was obtained from a number of different orders and families.[2] More recently evidence of another sort has been accumulating. Males of the variegated South American plover, *Vanellus chilensis*,[3] the pigeon and the guinea fowl, in all of which the sexes are similar, have shown no plumage response to the injection of theelin, nor in the latter species to castration. The plumage of the female guinea fowl is likewise unaffected by ovariectomy.[4] The extensive studies of Keck[5] on the English sparrow have shown that the distinctive plumages of the male and female of this species are not influenced by castration or by gonad transplantation.

These results indicate that the basis for the manifestation of sexual differences in plumage is not the same in all species. It apparently may differ radically within the same family (*e. g., Fringillidae*),[2, 5] when genera and even species may show interesting deviations,[6, 7] some of which suggest those found in breeds of poultry.

The experiments reported at this time were carried out on one of the *Icteridae,* the common Brewer's blackbird *(Euphagus cyanocephalus)* of western North America. The birds were captured with a large net while roosting at night, and were kept during the period of observation in large screened yards with adequate shelter. The tests were made in the late fall of 1933, about 5 months after the end of the breeding season. The sexes in this species differ in color of the plumage and in color of the iris. Adult males are of a rich iridescent blue-black over the head, neck, breast, and anterior part of the body generally, while the corresponding regions of the female are of a dark brown or rusty black with slight iridescence. The iris of the male is very pale yellow, approaching white; that of

[1] Danforth, C. H., *J. Exp. Zool.*, 1933, **65**, 183.
[2] Morgan, T. H., Carnegie Inst. Wash. Pub. 285, 1919.
[3] Vinals, Eduardo, *Comp. rend. Soc. biol.*, 1932, **109**, 1332.
[4] Hardesty, Mary, *Anat. Rec.*, 1934, **50**, suppl., 55.
[5] Keck, Warren N., *J. Exp. Zool.*, 1934, **67**, 315.
[6] Mayr, Ernst, *Am. Mus. Novitates* No. 714, 1934.
[7] Keck, W. N., and Witschi, Emil, *Anat. Rec.*, 1933, **57**, suppl., 28.

the female a rich dark brown. The male weighs about 70 gm., the female about 60 gm. Only males were used in these experiments.

Experiment I. On Nov. 8 feathers were plucked from regions on the head and breasts of 2 adult males. From Nov. 16 to Nov. 24, inclusive, each of these males received daily by injection into the pectoral musculature 2 cc. or about 100 rat units of theelin, the standard Parke Davis preparation being used. This was a total of 18 cc. or 900 rat units for each bird.

Experiment II. On Nov. 8, two adult males were prepared as above, and from Nov. 16 to Nov. 24 each of these was given 0.8 mg. daily of thyroxin by mouth. Squibb's thyroxin tablets were used.

Experiment III. On Dec. 12, two other males that had been similarly prepared were each given 3 cc. or 150 rat units, of theelin, and on Dec. 13, 14 and 15, 4 cc. daily in 2 injections, morning and night, in order to keep the concentration in the blood continuously high. On Dec. 16, each was given 3.5 cc. This is a total of 18.5 cc. or 925 rat units, of theelin to each bird within a period of 5 days.

Careful examination with the naked eye and hand lens revealed no changes in the color of the regenerating feathers or the irises of any of these treated specimens. The amount of theelin administered each day in Experiment I was, with reference to body weight about 3 times the amount that had been found to produce a "feminized" area in regenerating feathers of a male fowl following only 2 injections. Stated differently, if the male blackbirds had shown a response comparable to that of the fowl, per gram of body weight, the theelin injected into each bird would have been sufficient to produce detectible feminization of the regenerating feathers in at least a dozen specimens. In Experiment III the dosage for the 3 middle days was double the daily dose in Experiment I. Similarly, the daily thyroxin dosage was, in proportion to weight, over 6 times as great and was continued 3 times as long as would be necessary to produce marked effect in a male fowl. The experiments, therefore, may be regarded as entirely negative, indicating that at least in the season when the tests were made the reagents used do not exercise an inhibitory influence on the male coloring of either the plumage or the iris. Since in nature the birds show no seasonal changes in regard to these traits, it seems unlikely that they would show seasonal differences in response to the hormones.

In conclusion, the data thus far available indicate that in the male Brewer's blackbird, as in the male English sparrow, the color of the plumage is not subject to modification by the female hormone or by thyroxin. This brings to light another form in which the pattern

of endocrine-plumage relationship differs from that which was at first thought to be general. In these blackbirds the eye color also proves to be resistant to the effects of theelin and thyroxin.

7815 C

Some Effects of Alpha Dinitrophenol on Pregnancy in the White Rat.*

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It is a generally accepted fact that alpha dinitrophenol, when given in sufficient amounts, will raise the basal metabolic level and reduce weight.[1,2] There is sufficient proof that this action differs materially from that of thyroid,[3,7] and under ordinary circumstances does not damage kidneys or liver.[2,4] In spite of a raised metabolic level,[5,6] weight reduction does not occur when the caloric intake is increased above the individual requirement. We have been curious to learn what effects this drug might have on fertility, gestation, and fetal life. We therefore have studied this problem with the following points in mind: (1) Ability to become pregnant, (2) body weight changes during pregnancy and lactation, (3) Number and weights of young born, (4) Effect on suckling young, (5) number and weights of young reared.

Thirty-four female rats were studied in 3 groups:

Group 1.—Nine females were caged for 8 days for observation prior to the addition of males. This group received no treatment.

Group 2.—Five females were given 10 mg. of 1% aqueous solu-

* Supported by a grant from the Rockefeller Fluid Research Fund of the Stanford University School of Medicine.

1 Tainter, M. L., Stockton, A. B., and Cutting, W. C., *J. Am. Med. Assn.*, 1933, **101**, 1472.

2 Tainter, M. L., Cutting, W. C., and Stockton, A. B., *Am. J. Public Health*, 1934, **24**, 1045.

3 Cutting, C. C., and Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 97.

4 Dunlop, D. M., *Brit. Med. J.*, 1934, **1**, 524.

5 Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1161.

6 Looney, J. M., and Hoskins, R. G., *New Eng. J. of Med.*, 1934, **210**, 1206.

7 Cutting, W. C., Rytand, D. A., and Tainter, M. L., *J. Clin. Invest.*, 1934, **13**, 547.

tion of sodium bicarbonate per kg. of body weight for the duration of the experiment. After the first 8 days males were introduced.

Group 3.—Twenty females were given 20 mg. of alpha dinitrophenol per kg. of body weight, administered as a 2% solution in 1% aqueous sodium bicarbonate. Males were introduced after the eighth day of the experiment.

All solutions were administered intragastrically twice daily until the respective litters were weaned. Cage temperature was maintained at a reasonably constant level. Food and water were supplied in abundance. Males were changed at intervals to insure successful breeding. Mothers were isolated as soon as pregnancy could be determined. All females and litters were weighed daily until the litters were weaned. The results are summarized in Table I, in which Groups 1 and 2 are unified for comparison with Group 3.

TABLE I.

	Group 1 and Group 2 Control and Bicarbonate	Group 3 Dinitrophenol Treated
No. Females Bred	14	20
No. Females Littering	14	18
Total Young Born	88	108
No. Young Born Alive	82	81
% of Young Stillborn	7% (6 rats)	25% (27 rats)
Aver. No. Young Born per Litter	6.3	6.0
Aver. No. Young Reared per Litter	5.07	3.01
% Mortality of Viable Young	13.4% (11 rats)	30.9% (25 rats)
% Viable Young Reared	86.6% (71 rats)	69.1% (56 rats)
Aver. Wt. of Young when Born	5.38 gm.	4.97 gm.
Aver. Wt. of Young when Weaned	24.23 gm.	27.19 gm.
Mother Aver. Body Weight Changes:		
Prepregnant to Littering	16.0 gm. Gain	17.0 gm. Gain
Littering to Weaning	28.1 gm. Loss	3.7 gm. Loss

Dinitrophenol-treated animals littered in 18 instances and 2 not littering were found to have a uterine infection common to breeding females. All control and bicarbonate-treated mothers littered. During the period of gestation, dinitrophenol-treated mothers gained an average of 17 gm. each as compared with 16 gm. each for the controls. In the period of lactation the dinitrophenol-treated mothers lost an average of 3.7 gm. each as compared with a loss of 28.1 gm. each in the controls.

The dissimilarity in loss of weight during the nursing period can be explained only in part on the basis of the average number of young nursed by each mother, *i. e.*, 5.07 in the control to 3.01 in the dinitrophenol group. Even if making a theoretical allowance for

this difference in favor of the control group, the discrepancy in weight loss in the 2 groups is considerable and demands further study.

The average number born in each litter was not affected by the use of dinitrophenol. For the dinitrophenol-treated mothers it was 6, while for the control group it was 6.28.

The average weight of young when born was 4.97 gm. each for the dinitrophenol group as compared with 5.38 gm. each for the control group. In the dinitrophenol-treated group, 25% of the total number of rats were stillborn, and in the control group only 6.81% were stillborn. Of the young reared to weaning, the average weight of those of the dinitrophenol group was considerably greater than that of the young of the controls.

It is of considerable interest that the mortality during the nursing period of the viable young born to mothers of the dinitrophenol group was 30.9%, as compared with 13.4% for the young of the control litters. We do not believe that the explanation rests with a disturbed milk supply. At the end of the nursing period the weight of the dinitrophenol young was greater than that of the controls, although the litters of the former were considerably smaller. Therefore, the available milk supply must have been abundant. We offer 2 explanations: (1) Dinitrophenol mothers neglect their young while in a febrile state, and only the more vigorous of the offspring manage to reach the mother for nursing. (2) A toxic agent is passed to the young through the milk, but as yet, we have no proof for this assumption.

Summary. (1) Dinitrophenol administered intragastrically twice daily, in 20 mg. per kg. of body weight did not appreciably affect the fertility of white rats. (2) This dosage of dinitrophenol in the presence of an unlimited diet did not appreciably affect the body weight gains of mothers during pregnancy. (3) Neither did it affect the average number of young born to each mother. Rats given dinitrophenol intragastrically will bear about as many young per litter as control rats, but the number of stillbirths is increased 3½ times over that of the control litters. (4) During the nursing period the mortality of the young of dinitrophenol-treated mothers was considerably greater than that of the young of control mothers.

7816 C

Glutathione Concentration of Livers and Muscles of Rats Following Injection of Hypophyseal Growth Hormone.*

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We have previously reported[1] that there is an increase in concentration of glutathione in muscles of mature rats after 15 daily injections of a potent growth hormone obtained by alkaline extraction of beef pituitary. In control animals similarly treated with a heat inactivated solution of the hormone we found no such change. Analyses of the livers showed no significant change. However, if animals were sacrificed a few hours after a single injection of the potent hormone we found a decided fall in the glutathione concentration of the liver with only a slight change, if any, in the muscle glutathione. We have no explanation to offer for this sudden change in the liver but believe that it may furnish a method for estimation of the potency of the growth hormone that is more rapid than the usual method by measuring growth after 20 days of injection. It may prove to have a more important significance since by its use the fundamental biochemical reactions induced by the growth hormone may be studied quantitatively and thus it may be possible to obtain additional information concerning the chemical reactions involved in the synthesis of tissues.

Mature female rats which had reached growth stasis normally were used. They were divided into 2 groups matched for weight. One group, the experimental, received a single injection of a growth hormone solution prepared according to the method of Evans.[2] In a previous trial daily injections of 1 cc. of this solution had induced an average growth of 50 gm. in 15 days in mature female rats. The control group received the same amount of the heat inactivated hormone. Both groups were injected then allowed to fast for periods varying from 8 hours to 24 hours, when they were killed. The livers and musculature of the posterior legs and loins were then quickly removed and prepared for analysis according to the method which we reported previously.[1] Glutathione was determined in the tungstic acid filtrate by iodometric titration after ascorbic acid had

* This investigation was aided by a grant from the National Research Council for the study of glutathione in relation to growth and hereditary size.

[1] Gregory, P. W., and Goss, Harold, *J. Exp. Zool.*, 1934, **69**, 13.

[2] Evans, H. M., *et al.*, *Memoirs Univ. Calif.*, 1933, **11**, 1.

TABLE I.

Glutathione Concentration in Livers and Muscles. Adult Rats After Injection of Potent and Killed Growth Hormone Solution

Exp. No.	cc. Hormone†	No. rats used	Killed after 1st injection	Liver GSH mg. %	Liver S.D	Liver Groups compared	Liver D / P. E.	Muscle GSH mg. %	Muscle S.D	Muscle Groups compared	Muscle D / P. E.
Normal Females.											
1	2 KGH	15	8 hr.	127.5±4.9	27.5	1-2	7.4	33.2±0.5	2.6	1-2	1.6
2	2 GH	15	8 "	83.1±3.3	21.6			31.6±0.8	4.6		
3	2 KGH then food	8	8 "	145.4±4.7	18.7	3-4	8.1	41.3			
4	2 GH then food	7	8 "	98.6±3.1	11.2			37.7			
5	2 KGH	15	12 "	125.3±3.8	21.1	5-6	16	33.3±0.4	2.4	5-6	2.2
6	2 GH	14	12 "	55.5±2.0	10.9			31.5±0.7	3.8		
7	1 KGH daily + food	16	5 days	144.8±3.7	21.4	7-8	3.2	34.6±0.6	3.2	7-8	6.4
8	1 GH daily + food	14	5 "	127.8±3.8	20.5			40.0±0.6			
Spayed Females.											
9	2 KGH	12	8 hr.	100.1±4.9	24.2	9-10	5.9	32.5			
10	2 GH	11	8 "	69.5±1.6	7.3			30.7			
11	2 GH then food	12	8 "	99.8±2.7	13.5			41.1			
12	2 GH	10	12 "	78.0±2.6	11.6						
13	2 KGH	15	24 "	152.6±3.7	20.4	13-14	14	34.3±0.4	2.4	13-14	4.2
14	2 GH	15	24 "	88.0±2.8	15.9			30.5±0.8	4.4		

† All animals except where noted were fasted after receiving the injection. GH refers to the potent growth hormone while KGH refers to the heated control solution in which the growth hormone was destroyed.

been removed by oxidation with 2,6-dichlorophenolindophenol. Details of the method are given in the paper referred to above.

Results are given in Table I. Besides the normal females we had at our disposal 75 mature female rats which had been spayed. These animals had reached their growth plateau normally and were suitable for this study. These results are also included in Table I. The potent hormone solution is designated "GH" and the heat inactivated solution as "KGH".

As early as 8 hours after a single injection the liver GSH concentration falls about 30%. At 12 hours the decrease amounts to about 55%. Similar decreases are found among the spayed rats although the data are not so complete. In groups 13 and 14, fasted for 24 hours after a single injection, the concentration of GSH in the livers of the experimental animals is still low. In experiments 3, 4, and 11 the animals were allowed food after the single injection. The presence of food seems to raise the GSH concentration of both liver and muscles.

If instead of a single injection the animals receive 5 daily injections of 1 cc. each and are killed 8 hours after the fifth injection we find a reversal of the concentrations. The liver GSH has returned to about normal while the muscle glutathione of the experimental group has increased to approximately the same level as we had previously found after 15 daily injections.

Lee and Schaffer[3] reported that the first rapid gain in weight following treatment with growth hormone was due in part to an increase in the water content of the tissue. We have made moisture determinations on a large number of livers and muscles from both normal and spayed females. It is interesting to note that there was a small but significant decrease in the moisture content of the livers of the potent hormone injected group. There was no significant difference in the moisture content of the muscles.

Further investigations are necessary to determine the cause of the decrease or increase of glutathione concentration following treatment with hypophyseal growth hormone. These results firmly establish an intimate correlation between the hormone and changes in the glutathione concentration of the tissues concurrently with the stimulation of increase in weight.

[3] Lee, Milton O., and Schaffer, Norwood K., *J. Nutrition*, 1934, **7**, 337.

7817 P

Proteolytic Enzymes of Monocytic and Polymorphonuclear Pleural Exudates.

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During inflammation an increase in the permeability of the blood vessels permits the passage of erythrocytes, leucocytes, serum proteins and fibrinogen. The digestion and absorption of these elements is accomplished by the proteolytic enzymes of the exudate. The simultaneous presence of substances which inhibit proteolysis suggests a study of their behavior, as well as of the rôle of activators and accelerators of enzyme action during inflammation.

The present contribution is an extension of Opie's observations[1] on the proteolytic enzymes of monocytic and P.M.N. exudates. This material was obtained by injecting mineral oil[2] or an aleuronat-starch mixture[3] into the pleural cavity of rabbits. After separation of the cells from the liquid portion of the exudate, they were washed with saline and extracted in distilled water. Proteolytic activity was then measured during autolysis or during digestion of gelatin by Northrop's[4] formol titration method.

It was observed that whereas monocytes contain only one proteinase, pepsin, which is active from pH 2.0 to 5.0, the optimum being at 3.0, the P.M.N. have pepsin, cathepsin and trypsin with optima at pH 3.0, 5.4, and 8.0, respectively. The serous portions of the exudates (S.F.) also differ in that the monocytic type contains a substance inhibiting peptic digestion by the leucocytes, while the P.M.N. enhances this activity. The S.F. of a P.M.N. exudate inhibits the tryptic activity of the corresponding cells, while cells of a monocytic exudate inhibit the tryptic activity of their S.F.

There is also an inhibitory mechanism which concerns the leucocytes themselves. This was first observed by Willstätter, Bamann and Rohdewald,[5] who showed the presence of mutually antagonistic extractable or "lyo"-enzyme and bound or "desmo"-enzyme in the

[1] Opie, E. L., *Physiol. Rev.*, 1922, **2**, 552.

[2] Lucke, B., Strumia, M., Mudd, S., McCutcheon, M., and Mudd, E. B. H., *J. Immun.*, 1933, **24**, 455.

[3] Gay, F. P., and Clark, A. R., *Arch. Path.*, 1926, **1**, 847.

[4] Northrop, J. H., *J. Gen. Physiol.*, 1926, **9**, 767.

[5] Willstätter, R., Bamann, E., and Rohdewald, M., *Z. f. Physiol. Chem.*, 1932, **204**, 181.

W.B.C. This was confirmed and their presence in both monocytes and P.M.N. cells of inflammatory exudates was demonstrated.

When gelatin was digested by the S.F. of either a P.M.N. or monocytic exudate, there was a decrease in carboxylic groups as evidenced by negative formol titration values between pH 4.5 and 5.5 (or 6.0). A similar phenomenon was observed during the digestion of a dipeptid, leucyl-glycine by the S.F. of the monocytic type. (Table I.) In view of the work of several authors[6, 7, 8]

TABLE I.

Hydrolysis of gelatin and leucyl-glycine by supernatant fluids of inflammatory exudates.

Initial pH	Corrected Formol Titration Values		
	Monocytic		P.M.N.
	Gelatin	Leucyl-glycine	Gelatin
3.0	0.02	—0.03	0.29
5.0	—0.74		—0.99
5.5		—1.61	
6.0	—0.44		0.14
8.0	0.54		3.44
8.5		2.90	

this is suggestive of resynthesis of the protein-split products present in the exudate due to a reversal of catheptic action. Whether a similar process may, under proper conditions, go on *in vivo,* during an inflammatory process, is under investigation. Should this phenomenon occur, it might help to explain some phases of the problem of delayed resolution.

A delay in separation of an exudate into its constituents (cells and S.F.) causes an inactivation of the trypsin inhibitor or a release or activation of trypsin. It also makes conditions unfavorable for resynthesis. Gay and Clark[3] showed that a S.F. loses its bactericidal power if it is permitted to stay in contact with its cells for several hours before being tested. The question naturally arises, do conditions which decrease bactericidal action also favor resynthesis in an inflammatory exudate, and hence delayed resolution? This remains to be determined.

[6] Wasteneys, H., and Borsook, H., *Physiol. Rev.*, 1930, **10**, 110.

[7] Voegtlin, C., Maver, M. E., and Johnson, J. M., *J. Pharm. Exp. Ther.*, 1933, **48**, 241.

[8] Blagowestschenski, A. W., and Jeremejew, G. W., *Biochem. Z.*, 1934, **270**, 66.

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7818 C

Effect of Eserine and Acetylcholine on Gastro-intestinal Motility in Normal Dogs.*

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Eserine (physostigmine) has been extensively employed both in medicine and in physiology to stimulate intestinal motility. Choline and its esters have been utilized for the same purpose, but with less satisfactory results. Dale[1] and his coworkers originally described the effect of acetylcholine and several of its esters upon gastro-intestinal motility. Magnus and Le Heux[2] considered choline as the hormone which induces intestinal motility and subsequently, numerous attempts were made to treat paralytic ileus with choline and acetylcholine. Matthes[3] and Loewi and Engelhart[4] report the effect of acetylcholine, even in larger doses, as evanescent, for the compound is rapidly destroyed in blood by an esterase, which, however, may be successfully inhibited by eserine, even in dilution of 1:40,000,000. In the presence of eserine, acetylcholine remains active and potent in the circulating blood. Eserine is specific for the inhibition of this esterase, and with this datum as point of departure, we investigated the physiologic effects of a combination of acetylcholine and eserine upon post-operative ileus. In the course of that work, it soon became apparent that the literature furnished inadequate description of the effect of eserine upon gastro-intestinal

* Aided by the Louis L. Cohen Fund.

1 Dale, H. H., *J. Pharmacol.*, 1914, **6**, 147.

2 Le Heux, J. W., and coworkers, *Pflüg. Arch.*, 1919, **173**, 8, and following volumes.

3 Matthes, K., *J. Physiol.*, 1930, **70**, 338.

4 Loewi, O., and Engelhart, E., *Arch. f. exper. Path. u. Pharmak.*, 1930, **150**, 1.

motility in the normal dog, and nothing whatever concerning the intestinal motor response of the normal dog to eserine-acetylcholine. This paper presents such findings in normal dogs. The effects of acetylcholine-eserine upon the paralytic ileus of peritonitis will soon be published. To our knowledge, the combination of acetylcholine and eserine has not been used clinically to stimulate motility of the alimentary tract.

Dogs, averaging 25 lb. in weight, were tested under ether anesthesia. Blood pressure was registered in the usual way. Rubber balloons introduced in the stomach, small intestine, and colon, were connected to water manometers. Body temperature was maintained by a heated operating table.

Eserine salicylate and acetylcholine (Hoffman-La-Roche) were given intramuscularly. An average control period of three-quarters of an hour preceded each injection, which was administered when tonus and peristalsis were regular and minimal.

Four dogs each received 0.5 mg. of eserine, 12 dogs received 17 injections of 1 mg. of eserine, 4 dogs were each given 2 mg. of eserine, and one dog, 3 mg. All drugs were given intramuscularly. It was soon evident that quantities less than one mg. frequently did not produce any motility, and doses above 1.5 mg. often caused undesirable concomitant effects such as excessive salivation, increased respiration, and a tetanus-like intestinal contraction. One mg. of eserine did not yield these undesirable effects, and although not always followed by peristalsis of the gastro-intestinal tract, yet this dose in combination with acetylcholine was sufficient to elicit peristalsis quite regularly.

The intramuscular injection of 0.025 mg. acetylcholine had no effect on blood pressure or intestinal motility, but combined with one mg. of eserine, produced in several dogs a slight drop in blood pressure of 10-15 mm. of mercury, which disappeared after 2 to 3 minutes.

From Table I, it is evident that one mg. of eserine had evoked gastric motility in only 50% of the tests, whereas the combination

TABLE I.

Effect of Intramuscular Injection of Eserine (1 mg.) and Eserine (1 mg.) plus Acetylcholine (0.025 mg.) on Gastro-Intestinal Motility.

	Stomach		Ileum		Colon	
	Eserine	Eserine + Acet. Chol.	Eserine	Eserine + Acet. Chol.	Eserine	Eserine + Acet. Chol.
Increased Peristalsis	8	12	6	11	10	13
No effect	8	2	10	3	6	1

of this quantity of eserine with .025 mg. acetylcholine produced increased peristalsis of the stomach in 12 out of 14 trials. Eserine had no effect on the ileum in 10 out of 16 tests, but eserine-acetylcholine was without effect in only 3 of a total of 14 experiments. On the colon, eserine alone was ineffective in 6 out of 16 tests; the combination of eserine and acetylcholine, in only one among 14 trials.

To appreciate better the distinction between eserine alone and the combination eserine-acetylcholine, we might mention that, generally speaking, the intestinal motor response elicited by the latter is more pronounced, more decisive, and more generalized. In dogs in which eserine evoked no reaction, or at best, slight colonic movement, eserine-acetylcholine yielded widely disseminated motility of the entire gut.

The average latent period after eserine was 12 minutes for the stomach, 18 minutes for the ileum, and 11 minutes for the colon. For eserine-acetylcholine, the latent period was 14, 10, and 9 minutes respectively. The average duration in the effect of eserine on stomach, ileum, and colon was 21, 26, and 32 minutes respectively, as compared with an average duration after eserine-acetylcholine of 28, 35, and 34 minutes respectively.

From the above results, it is evident that 1 mg. of eserine, when given intramuscularly into normal dogs of 25 lb. average weight, does not regularly produce peristalsis. In several experiments, eserine proved completely ineffectual; in other instances, motility was manifest in only one or 2 segments of gut. If one mg. of eserine is combined with 0.025 mg. of acetylcholine, a rather constant effect is obtained on stomach, ileum and colon. Although it is known that acetylcholine *in vitro* induces very strong peristaltic activity of the gut and that a great deal of this effect is preserved *in vivo* whenever eserine is present to inactivate the blood esterase, we do not claim that the results of eserine-acetylcholine on intestinal motility are due only to the preservation of the acetylcholine by eserine. The effect must be considered as the resultant of the stimulatory action of both eserine and acetylcholine. Larger doses of eserine (2-3 mg.) rather consistently stimulate extensive intestinal motility. However, in this work, our primary concern has been to determine that dose of eserine which not only preserves acetylcholine in the blood, but avoids as well any undesirable concomitant effects.

Summary. Twenty dogs, under ether anesthesia were injected intramuscularly with eserine and eserine plus acetylcholine. One mg. of eserine or less did not regularly produce peristalsis of the stomach, ileum, and colon. When 0.025 mg. of acetylcholine was

combined with one mg. of eserine, peristalsis resulted, almost constantly in all 3 viscera. Intramuscular injection of larger amounts, 2-3 mg., of eserine regularly induced generalized intestinal motility but caused undesirable concomitant effects as well.

7819 P

Virus Adsorbed to Alumina-gel for Production of Antipoliomyelitis Serum in Sheep.

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Early in the history of experimental poliomyelitis it was demonstrated that the serum of both monkeys and humans, convalescent from the disease, had the property of neutralizing the action of the virus when the 2 were mixed *in vitro*. As therapeutic value has been ascribed to such serums a number of attempts have been made to produce antiserum with similar properties in large, poliomyelitis-refractory animals. In most instances horses have been employed and the results, although sometimes successful, have been irregular and seem to depend upon individual differences in responsiveness of the animals. The immunization of sheep has met with success in some hands (Howitt[1]) and failure or irregular results in others (Stewart and Haselbauer[2]). The production of poliomyelitis antiserum in goats has also been reported.

In these various types of animals, virus inoculations have usually been continued over a long period of time, in some instances years, before potent neutralizing serums were demonstrated. A method of immunization in which the time as well as the number of injections could be decreased would be a distinct improvement.

We were subjecting a sheep to a series of virus injections for the purpose of producing an antiserum when it occurred to us that virus adsorbed to a colloidal carrier might prove to be a better immunizing agent than the crude virus. There is evidence that the antigenicity of substances may be enhanced by injecting them in combination with a colloid. Hektoen and Welker[3] have reported the continued maintenance of a high precipitin level in rabbits following one injection of protein adsorbed to alumina-gel. Since the virus of

[1] Howitt, B. F., *J. Inf. Dis.*, 1932, **50**, 26.

[2] Stewart, F. D., and Haselbauer, P., *J. Exp. Med.*, 1928, **48**, 449.

[3] Hektoen, L., and Welker, W. H., *J. Inf. Dis.*, 1933, **53**, 309.

poliomyelitis has been adsorbed to this substance by Sabin[4] and others, we decided to investigate the suitability of such a combination as an immunizing agent in sheep. Virus-alumina-gel complex has been used as an immunizing agent for susceptible animals in poliomyelitis by Rhoads[5] and by us.[6] It is perhaps noteworthy that 2 horses which produced good neutralizing serum within 5 months were injected with a preparation in which 0.1% alum had been incorporated in the saline (Weyer, Park and Banzhaf[7]).

Our sheep (L) had been given 16 weekly injections of crude virus over a period of 4 months. After a rest period of 7 weeks, 2 injections of virus-alumina-gel complex were given with an interval of 3 weeks. The crude virus injections consisted of pooled glycerolated 10% virus given both subcutaneously and intramuscularly, usually 50 cc. by each route. The virus-alumina-gel was prepared by shaking together equal parts of ether-treated virus, at a pH of 6.5, and alumina-gel (Willstätter, Type C). The first injection consisted of 50 cc. and the second of 65 cc., both given subcutaneously.

TABLE I.

Production of poliomyelitis antiserum in sheep.

Treatment	Blood collected	Serum Neutralization Tests		
		Dilution of serum	Sheep L Virus injection	Sheep R normal tissue injection
——	Before injections	undiluted	—	—, —, —
16 weekly injections of crude material	3 weeks after last injection	"	+	—
2 injections of gel preparation	1 month after last injection	"	+	+, —
		1:5	+	—, —
		1:25	+	—
		1:50	+	
		1:100	+	
	5 months after last injection	undiluted		+, —
		1:5		—, —
		1:25	+	+, —
		1:50	+	—
		1:100	+	
		1:250	+	
		1:500	+	

+ indicates neutralization; —, lack of neutralization. Each sign represents one monkey.

[4] Sabin, A. B., *J. Exp. Med.*, 1932, **56**, 307.

[5] Rhoads, C. P., *J. Exp. Med.*, 1931, **53**, 399.

[6] Gordon, F. B., Harrison, J. A., and Hudson, N. P., paper read before Soc. Am. Bact., Dec. 29, 1934.

[7] Weyer, E. R., Park, W. H., and Banzhaf, E. J., *J. Exp. Med.*, 1931, **53**, 553.

As a control, another sheep (R) had been given parallel injections of a crude emulsion of the central nervous system of normal monkeys. It also was given 2 similar injections of alumina-gel which had been treated with nervous tissue from normal monkeys in the same manner that the virus-alumina-gel was prepared. Table I gives the results of neutralization tests made with serum from different bleedings of both sheep.

Attention should be called to the fact that we have not found an end-point in the serum titre of the sheep (L) injected with virus. In every instance neutralization was effected by the highest dilution of serum used, which in one instance was 1:500. We were gratified to find that the serum possessed good neutralizing power 5 months after the last immunizing injection. Irregular results were obtained with the serum of the sheep (R) that received normal nervous tissue. This is consistent with the reports of several workers that normal sheep serum may in some cases neutralize the virus, and that this property may fluctuate in individual sheep.[2]

7820 P

Mechanism of Death in Bile Peritonitis.

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The question of the mechanism of death in so-called "bile peritonitis" due to the leakage of moderate or large amounts of bile into the peritoneal cavity has puzzled investigators for some time. The resultant peritonitis has been attributed to a toxic action or the action of anaerobic organisms. Mason[1] and Andrews[2] noted that a large amount of exudate was formed in the peritoneal cavity of animals dying from liver autolysis and experimentally produced bile peritonitis. Blalock,[3] Underhill,[4] Harkins,[5] and others have shown that

[1] Mason, E. C., and Davidson, E. C., *J. Lab. and Clin. Med.*, 1925, **10**, 622.

[2] Andrews, E., Rewbridge, A. G., and Hrdina, Leo, *Surg. Gynec. and Obst.*, 1931, **53**, 176.

[3] Beard, J. W., and Blalock, A., *Arch. Surg.*, 1931, **22**, 617.

[4] Underhill, F. P., Kapsinow, R., and Fisk, M. E., *Am. J. Physiol.*, 1930, **95**, 302.

[5] Harkins, H. N., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 994; and articles on freezing, in press.

TABLE I.

Blood concentration changes, blood pressure fall, chemical analysis of blood plasma and peritoneal fluid, and amount of peritoneal exudate found at necropsy in bile peritonitis produced by 3 different methods.

No.	Procedure	Hours before death	Wt. kg.	Time	Hb.	Hemat.	Blood Pressure mm. Hg.	NaCl mg./100 cc.		Total Protein gm./100 cc.		Peritoneal Exudate
								Blood	Fluid	Blood	Fluid	% body wt.
1	7.5 gm. bile salt/kg.	5	14.0	Start	93	52	150	656	615	4.55	3.87	2.5
				End	162	71	26					
2	" " " " "	6	8.5	Start	62	45	170	649	516	2.86	4.31	2.1
				End	135	66	46					
3	2.5 " " " "	22*	13.7	Start	107	47	182	623	645	3.92	4.15	1.6
				End	140	64	114					
4	" " " " "	5½*	15.5	Start	93	44	144	606	672	12.46	4.17	2.0
				End	138	71	82					
5	2 cc. whole bile/kg.	5	7.5	Start	97	44	156	—	625	—	4.00	1.1
				End	136	64	132					
6	" " " "	19½*	14.0	Start	73	37	190	664	683	4.63	3.65	1.5
				End	102	53	126					
7	" " " "	6	9.8	Start	92	44	130	647	—	3.57	—	1.2
				End	112	50	54					
8	" " " "	20*	12.2	Start	86	41	158	665	613	4.26	5.47	0.5
				End	110	51	132					
9	Common duct ligation and gall bladder defundation	30	9.0	Start	82	35	—	—	571	7.65	4.96	2.7
				End	—	—	—					
10	"	14	10.0	Start	83	40	144†	563	630	7.79	5.45	4.4
				End	110	54	64					
11	Control	25*	24.5	Start	86	48	130	636	—	6.56	—	—
				End	125	55	110					
12	"	24*	7.8	Start	103	53	140	669	—	5.42	—	—
				End	113	54	116					

* Denotes that the animal was killed by bleeding. All other animals died spontaneously.

† Blood pressure reading taken after ligation.

in burns, freezing, and intestinal trauma, a large factor in the production of the resultant shock and death is the loss of a large amount of plasma-like fluid from the blood stream. To test the hypothesis that the loss of a similar plasma-like fluid into the peritoneal cavity might be a large factor in the production of death in bile peritonitis, the present work was undertaken.

Bile peritonitis was produced by 3 methods: The intraperitoneal injection of sterile bile or bile salts, and ligation of the common duct followed by defundation of the gall bladder. In most instances the dogs used in these experiments died from 5 to 30 hours following the procedure. The results are shown in Table I. There was marked concentration of the blood with a greatly increased hemoglobin percentage and hematocrit reading and a marked fall in carotid blood pressure. Necropsy revealed an enormous collection of peritoneal fluid, amounting in several instances to over 3% of the body weight. This fluid clotted spontaneously and chemical analysis revealed that it conformed closely in amount of total protein, nonprotein nitrogen, sugar, and chloride content to the blood plasma of the animal in question. Cultures of the fluid revealed no constantly present organism.

Conclusion. The amount of plasma-like peritoneal exudate in experimental bile peritonitis indicates that the loss of this fluid from the blood stream is an important factor in the production of shock and death in this condition.

7821 P

Comparative Study of Respiratory Portion of the Lung.*

C. G. LOOSLI. (Introduced by W. Bloom.)

From Hull Anatomical Laboratory, University of Chicago.

All authors agree that the respiratory portions of lungs of Amphibia and Reptilia are lined by continuous, flattened, nucleated, epithelial membranes. Some investigators maintain that there is a continuous, flattened nucleated and non-nucleated epithelial membrane lining the respiratory portions of mammalian lungs, while others deny the presence of an epithelium. Some believe that the small, nucleated cells found in the meshes of the capillaries in the

* This research was aided by a grant to the University of Chicago by the Rockefeller Foundation.

alveolar septa are of mesenchymal origin. For a review of the literature on this subject see Fried.[1]

This work comprises a study made on the nature of the lining of frog, turtle, chicken, rabbit, guinea pig, and rat lungs. The lungs of the animals, after their lung capillaries had been filled with blood by ligating their pulmonary veins before the animals were killed, were fixed in Zenker-formol solution, embedded in nitrocellulose and stained to show collagenous, reticular and elastic fibers and cellular details. In addition, rabbit lungs, made atelectatic by phrenicotomy and pneumothorax were kept in a state of collapse from one to 40 days and were studied with the same histological methods. To demonstrate cell outlines, intratracheal and intravascular injections of 2% silver nitrate were also made into the lungs of other animals in each of the above groups.

A continuous layer of flattened, nucleated epithelium covering the alveolar surfaces in frog and turtle lungs is easily demonstrated both in the silver nitrate and Zenker-formol fixed preparations after they are stained respectively with hematoxylin eosin-azure and Mallory's phosphotungstic hematoxylin. The nuclei of the flattened cells are usually located in the meshes of the capillaries. Intratracheally silver-injected preparations show a system of black lines on the surfaces of the alveolar septa continuous with the limits of the cuboidal cells which line the main air passages. Intravascularly silvered lungs show an entirely different set of lines which do not cross the intercapillary spaces in the alveolar septa, but which are continuous with the lines which mark the limits of the endothelial cells of the blood vessels.

An epithelial membrane covering the capillaries is not demonstrable in the walls of the respiratory canaliculi of chick lungs. Intratracheally and intravascularly silvered preparations outline only the endothelium of the capillaries in the walls of the canaliculi.

Intratracheally and intravascularly silvered lungs of rabbits, guinea pigs, and rats show essentially the same features as the silvered chick lungs. The black, silver lines correspond to the limits of the capillary endothelium and follow the course of the capillaries from one side of a septum to the other. At the junction of the capillaries with the venules the black lines, following the capillaries in the septal walls, are continuous with the lines which mark the limits of the endothelial cells lining the veins. The septal cells are not delimited by the silver but remain isolated on the alveolar walls independent of the silvered lines. Non-nucleated plates were not seen.

[1] Fried, B. M., *Arch. Path.*, 1934, **17**, 76.

In completely collapsed, rabbit lungs, the lumina of the alveolar ducts, alveolar sacs and alveoli, the walls of which become shortened, thickened, and folded on themselves, are represented as narrow, branching clefts. At the junction of the terminal and respiratory bronchioles with the alveolar ducts, the bronchial epithelium and the reticular, basement membrane on which it rests cease abruptly. The connective tissue and blood vessels of the lamina propria of the smallest terminal and respiratory bronchioles continue on and form the walls of the alveolar ducts and alveoli. The free surfaces of the alveolar ducts between the mouths of the adjoining alveoli are covered with capillary loops, which are seen in thin sections to arise on one side of a septum and continue over the connective tissue knob and onto the adjacent septal wall.

Each alveolar septum contains a network of capillaries which course back and forth from one side of the septum to the other. The capillaries lie in the meshes of the reticular and elastic fiber networks and form loops which bulge into the almost obliterated, former, air spaces. The reticular and elastic fibers in and on which the capillaries rest are separated from the alveolar spaces by the capillary network. The septal cells do not form a continuous membrane over the capillary loops in the alveolar walls following collapse of the lung, but remain in the intercapillary spaces intimately associated with the connective tissue as isolated cells. In the alveolar walls the capillaries with their reticular and elastic tissue support are enclosed in an amorphous, ground substance, which can be demonstrated with the Mallory-Azan stain. In short, a continuous, histological epithelium is not demonstrable in the respiratory portion of the completely collapsed, rabbit lung.

An embryogenic study of the rat lung is being made to determine the origin of the septal cells.

7822 P

Pathogenesis of "White Bile."*

HANS G. ARONSOHN. (Introduced by Edmund Andrews.)

From the Department of Surgery, The University of Chicago.

White bile is a relatively rare but clinically important condition on account of its grave prognostic significance. My own previous work on this subject (experimental production of white bile in

* Work done under a grant from the Douglas Smith Foundation.

rabbits) gave the following results; that one might expect to find it when there was present an active infection in the bile ducts, and that it was also dependent upon the activity of the liver cells.

Ligations of the common duct were made in the dog and 4 weeks later bacteria were injected into the bile tracts in order to see what influence infection had on the production of white bile.

Eleven dogs were operated upon of which 4 died from 2 to 20 days after the operation (Group 1). In 6 dogs 4 weeks later bacteria† were injected into the gall-bladder. One dog was kept as a control without the second operation. Three of the 6 dogs survived the second operation for only from one to 13 days (Group 2). The remaining 3 and the control dog remained alive and were sacrificed after 4½ to 5½ months (Group 3). All 3 dogs of Group 3 had white bile. In one dog of Group 2 there was very light colored bile. Both of the other dogs of Group 2, the control dog of Group 3 and all the dogs of Group 1 had frank green bile. Chemical analysis of bile in Group 3 showed a marked diminution or a complete lack of the main constituents of bile (bilirubin, cholesterol and bile salts). In the cases with white bile from Group 3, the following common characteristics were noted. There was the clinical picture of a severe infection lasting several months. The dogs were apparently very ill and bacteriologically there was a virulent infection in the bile accompanied by numerous leucocytes in the sediment. In the control dog these phenomena were absent. It is interesting that in Group 2 one of the dogs with all the signs of infection for 32 days had a bile that was very light colored while the other 2 dogs without signs of infection had green bile.

It appears, therefore, that we find white bile in the common duct in cases of obstruction which had been accompanied by severe infection after a considerable time. A review of the few cases in the literature in which the bile has been studied after relief of the common duct obstruction (Walters and Greene, Andrews[3]) gives further support to this theory. Clinically, much clear fluid is discharged from the fistula the first few days, to be replaced finally by colored bile from the liver. The presence of pigment in the liver and in the liver cells in generalized icterus speaks against the conception that this colorless bile has been produced by the liver cells

† *Streptococcus hemolyticus, Staphylococcus albus* and *S. aureus.*

[1] Andrews, Edmund, Hrdina, L., and Dostal, L. E., *Arch. Surg.*, 1932, **25**, 1081.

[2] Aronsohn, Hans G., *Bruns' Beitr. zur klin. Chir.*, 1932, **156**, 63.

[3] Walters, Waltman, Greene, Carl H., and Frederickson, Clyde H., *Ann. Surg.*, 1930, **91**, 686.

themselves. Furthermore it appears hardly conceivable that the function of the liver cells in a space of 6 to 8 days after relief of the obstruction could change itself from that of a colorless secretion to normal bile. My theory of the white bile in relation to the clinical observations noted after relief of the common duct obstruction is as follows: First, the decolorization of the stagnant bile in the bile duct is caused by infection. The liver cells produce for a considerable period after obstruction a green bile and this secretion is finally stopped on account of the rise in pressure in the liver or it may flow back into the blood stream. After the relief of the obstruction, the white bile already present in the dilated bile ducts pours out first. The fluid discharged in the first few days one might expect to be the secretion of the bile tract epithelium in which a further recurrent infection has been stirred up by the operation. Finally the liver function itself returns, provided that the liver cells have not been too severely damaged, and true bile appears.

7823 C

Thyroid Hypertrophy in the Rat with Reference to the Effect of Light.*

ALLAN T. KENYON. (Introduced by O. H. Robertson.)

From the Department of Medicine of the University of Chicago.

In describing the thyroid hypertrophy and colloid loss in rats exposed to cold and darkness for 10 to 25 days the writer[1] noted that he had not seen any striking changes due to darkness alone. Bergfeld[2] had previously stated that in 4 to 6 weeks, absence of ultraviolet light caused thyroid hypertrophy irrespective of the environmental temperature and that in his small series no effects attributable to cold were found if the room were illuminated. While students using the rat have not agreed on this effect of darkness, conclusive evidence that in the chick absence of ultraviolet light for 55 to 105 days results in marked thyroid hypertrophy and colloid loss has been provided by Turner and Benedict.[3] The interesting possibility thus suggested that light may play some vital rôle in

* This study was conducted in part under a grant from the Douglas Smith Foundation for Medical Research.

1 Kenyon, A. T., *Am. J. Path.*, 1933, **9**, 347.

2 Bergfeld, W., *Endokrinologie*, 1930, **6**, 269; *Strahlentherapie*, 1931, **39**, 245.

3 Turner, K. B., and Benedict, E. M., *J. Clin. Invest.*, 1932, **11**, 761.

the formation of the thyroid hormone has led the writer to further examine its possible influence under the conditions of temperature and diet used in his earlier work.

From March 8 to June 13, 1933, (98 days) 10 albino rats of about 90 gm. in weight were kept in a dark room at an average temperature of 26.5°C. and compared with 9 litter-mates of the same sex kept in ordinary room light at 25° and irradiated an average of 40 minutes weekly at 24 inches with an Alpine sun (Mercury vapor) lamp. During the last 75 days of this study 3 rats of about 60 gm. and 6 uncontrolled young, born in the dark, were added to the series. The diet (yellow corn 62; linseed oil meal 13; whole milk powder

TABLES.

Experiment	No. of Animals	Amount of Colloid: 0-1 most	0-1 part	0-1 absent	0-1, 2	2	2, 3	3	3, 4	Height of Epithelium: 3, 4	3	2, 3	2	1, 2	Aver. wt. of thyroid mg. / 100 gm. body wt.
1 a.		March 8 to June 13, 1933								98 days					
Dark at 26.5°	10	3	2	5	0	0	7	0	0	5	2	3	0	0	17.2
Light and irrad. at 25°	9	0	0	9	0	1	6	0	2	0	2	1	4	2	9.8
1 b.		April 1 to June 13, 1933								75 days					
Dark at 26.5°	3	0	0	3	0	0	2	1	0	0	0	0	3	0	11.9
Light and irrad. at 25°	3	0	0	3	0	0	3	0	0	0	0	0	3	0	9.8
Dark at 26.5° (Born in dark)	6	0	0	6	0	0	6	0	0	0	0	2	4	0	17.3
1 c.		January 15 to May 4, 1934								109 days					
Dark at 28.7°	22	1	2	19	1	1	15	4	0	3	5	7	7	0	10.4
Light and irrad. at 25°	20	1	5	14	4	0	14	0	1	5	10	3	2	0	12.4
2		March 21 to April 13, 1934								13 days					
Dark at —3°	16	5	8	3	8	0	3	0	0	10	5	1	0	0	17.0
Dark at —3° and irrad.	16	7	7	2	3	2	4	0	0	9	7	0	0	0	18.0
3 Cold		Aug. 28 to Sept. 8, 1934 (11 days)								Recov. to Sept. 20 (12 days)					
Dark at 29.5°	8	5	3	0	3	0	0	0	0	8	0	0	0	0	23.0
Dark and irrad. at 29.5°	8	2	6	0	4	0	2	0	0	6	2	0	0	0	24.0

Key: The first series of columns under "amount of colloid" designates the occurrence of areas of little or no colloid (0-1) throughout the gland, in parts, or absent. The second series of columns gives the distribution of various grades of follicular colloid content predominating in such glands as have more than traces of colloid; 2 = small follicles filled with colloid; 3 = medium follicles filled with colloid; 4 = large follicles filled with colloid. Such conjoint expressions as 0-1, 2 indicate that both degrees are found in abundance. Under "height of epithelium" 4 indicates high columnar; 3 = low columnar; 2 = cuboidal, and 1 = flat. Conjoint expressions as 3, 4 indicate that both types are present in large numbers.

18; casein 4; alfalfa 2; sodium chloride 0.5; and calcium carbonate 0.5) had an iodine content of 13.5 gamma/100 gm. (second month) and 15.6 gamma/100 gm. (third month). The significance of the colloid loss appearing in half of the group kept 98 days in the dark is unfortunately probably vitiated by the presence of pulmonary and liver infection disclosed at autopsy in both experimental and control animals, but is of interest in indicating that thyroid hypertrophy may occasionally occur in rats under certain circumstances on diets yielding 1.4 gamma of iodine daily. (Table 1 a, 1 b.)

From January 15 to May 5, 1934, (109 days) 22 rats of about 120 gm., and of a mixed strain, free from infection at the end, were kept in the dark at 28.7°C. and compared with 20 litter-mates of the same sex and hood-color kept at 25° and irradiated as before for an average of 27 minutes weekly. The diet had an iodine content of 6.9 gamma/100 gm. No striking hypertrophy and colloid loss occurred generally in the experimental animals although the occasional appearance of such changes, both in the experimental and control series, indicates that the stock as a whole should have been favorable for such a development. Mayerson and Branch[4] have recently reported a careful study in agreement with these findings (Table 1 c).

From March 20 to April 3, 1934, (13 days) 16 of 32 animals kept at —3°C. in the dark, were irradiated at 28° for 10 minutes on each of 8 days. The striking hypertrophy and colloid loss produced by cold are evident in both groups. This is as far as the writer's present physical set-up permits him to go in testing the possibility that ultraviolet light inhibits the changes induced by cold and the results are, within these limits, negative (Table 2).

From August 28 to September 8, 1934, (11 days) 16 animals were exposed to cold and dark and marked hypertrophy produced in all, as judged by biopsy. They were then placed in the dark at 29°C. and half of them irradiated 30 minutes daily for the last 10 days of their 12-day stay. The diet during both cold and warm periods (yellow corn 75; linseed oil meal 15; casein 5; alfalfa 2; brewer's yeast 2; sodium chloride 0.5; calcium carbonate 0.5) contained 8.7 gamma iodine/100 gm. The irradiation did not decisively influence the rate of recovery from the effects of cold (Table 3).

By the twelfth day in a warm room the recovery from the effects of the cold had progressed a little way only, as judged by recession

[4] Mayerson, H. S., and Branch, C. H. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 650.

of hypertrophy and accumulation of colloid. The intense mitotic proliferation of the biopsy specimens had, however, completely subsided. The diet containing 8.7 gamma per 100 gm. is unable, in the time given, to provide materials with which a substantial restoration of colloid can be achieved.

These experiments provide information as to the level of iodine intake at which cellular hypertrophy and colloid loss due, presumably to the processes of endemic goiter, may still be expected to occur. Thus the spontaneous changes in certain animals recorded in Table 1 c appeared with an iodine content of the diet of 6.9 gamma per 100 gm., while the darkened infected group of Table 1 a showed these changes on diets containing from 13.5 to 15.6 gamma per 100 gm.

In summary it may be observed that under the dietary and temperature conditions of the writer's work no decisive evidence was obtained that darkness induces marked colloid loss or cellular hypertrophy in the thyroid gland, nor was it possible by brief irradiation with ultraviolet light to influence unmistakably the hypertrophy induced by cold or the recovery therefrom. If it is true, as some have felt, that light is of great importance to the thyroid of the rat, the circumstances under which this influence can be satisfactorily established remain unknown.

The writer wishes to express his great obligation to Dr. Versa Cole of the Department of Surgery of the Ohio State University School of Medicine for the determinations of the iodine content of the foods used.

7824 P

Sex Difference in White Rat in Tolerance to Certain Barbiturates.

HARALD G. O. HOLCK AND MUNIR A. KANAN. (Introduced by A. J. Carlson.)

From the Department of Pharmacology, School of Medicine, American University of Beirut, Beirut, Syria.

Using duration of sleep and rate of mortality as criteria, we have verified the Nicholas and Barron[1] finding that the female white rat is much more sensitive to amytal* than is the male, in that on any

[1] Nicholas, J. S., and Barron, D. H., *J. Pharm. and Exp. Therap.*, 1932, **46**, 125.

* The drugs used were kindly furnished by the following firms: amytal, Eli Lilly & Co.; nembutal, Abbott Laboratories; evipan, Bayer Co.; pernocton, Riedel-de Haen, Inc.; hebaral, Parke, Davis & Co.

given, suitable dose it sleeps much longer and is more apt to die [18 males (m.), 18 females (f.)]. A similar sex difference is found with nembutal (15 m., 15 f.), contrary to Nicholas and Barron; to evipan or evipal (111 m., 86 f.), contrary to Kennedy[2]; to pernocton or pernoston (22 m., 22 f.), and, perhaps not quite as marked, to hebaral or ortal (15 m., 15 f.). Thus, as an example, after administering 363 mg. of sodium evipan per kilo, subcutaneously, to 5 winter males (summer much more sensitive), these sat up perfectly in from 1.0 to 3.5 hours; 4 females sat up in from 8.1 to 10.1 hours, and one died on this dose. No such sex difference could be found with barbital (8 m., 8 f.), or phenobarbital (8 m., 8 f.) In case of pernocton some rats die on the 2nd or 3d day, which agrees with the report by Barlow[3] and his associates and with the delayed deaths noted with the chemically related noctal by Fitch and Tatum.[4]

No sex difference to evipan could be detected in the dog (15 m., 15 f.), cat (8 m., 5 f.), rabbit (6 m., 4 f.), guinea pig (9 m., 9 f.), white mouse (10 m., 10 f.), turtle (10 m., 10 f.), or frog (20 m., 20 f.). This agrees with the Fitch and Tatum report upon rabbit sensitivity to barbiturates in general, with Kennedy in regard to white mice, and with our own study[5] upon amytal in the dog and rabbit, in case of which latter it had even appeared that the females recovered more promptly than the males, though the mortality was about even for the 2 sexes.

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[2] Kennedy, W. P., *J. Pharm. and Exp. Therap.*, 1934, **50**, 347.

[3] Barlow, O. W., ———, *Anesth. and Analg.*, 1931, **10**, 251.

[4] Fitch, R. H., and Tatum, A. L., *J. Pharm. and Exp. Therap.*, 1932, **44**, 325.

[5] Holck, H. G. O., and Kanân, M. A., *J. Lab. Clin. Med.*, 1934, **19**, 1191.

Southern California Section

Scripps Institution of Oceanography, La Jolla, Calif., December 28, 1934.

7825 P

Determination of pH of Living Tissue by the Glass Electrode.*

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From the Department of Biological Sciences, University of California at Los Angeles.

The ordinary bulb type of glass electrode is necessarily limited in its use for determining the hydrogen-ion concentration of *living* tissues to fluids or to cavities in which such an instrument can be placed. For example, the pH of the blood can be determined by actual insertion of the electrode in some of the larger vessels, or the blood stream can be diverted into a chamber surrounding the glass bulb. Other determinations of the pH of living tissue have involved the actual destruction of that tissue, regardless of whether the colorimetric method, or the hydrogen, the quinhydrone, or the glass electrode was used. We have employed a spear type of capillary glass electrode which can be inserted directly into the tissues; in this series of experiments, into the intestinal wall of the white rat. The vacuum tube amplifying system used was essentially that described by DeEds.[1] The electrodes were drawn of Corning No. .015 glass, and were from 30μ to 100μ in thickness, the capillary wall varying from 10μ to 30μ. They were filled with 1/10 N HCl saturated with quinhydrone, sealed at the tip, and calibrated at the temperature of the tissue just before and immediately after using. The animal was completely insulated, being in contact only with the tip of the glass electrode and with the end of the calomel half cell. The latter was inserted into the peritoneal cavity of the rat, which was kept under ether anesthesia throughout the experiment; a por-

* Aided by a grant from the Board of Research, University of California.

[1] DeEds, F., *Science*, 1933, **78**, 556.

tion of the intestine was fastened to an upright slab of hard rubber; an incision was made in the wall of the gut and a reading taken at once, care being taken to prevent loss of heat. Readings were taken of both the contents and wall in different parts of the intestine. No difference could be demonstrated between readings taken at the surface of the intestinal mucosa and those obtained by dissecting away the surface layer and inserting the electrode directly into the deeper tissues of the gut wall. The galvanometer readings were exceedingly stable during the 2-3 minutes necessary to take an observation, and were not affected by action currents in the animal.

The greatest difficulty was to obtain electrodes which would give reproducible readings; in many cases the electrodes were too inconstant to be calibrated. These changes were spontaneous, and appeared to be independent of the so-called "deviation film" of Kahler and DeEds.[2]

The following readings were taken with electrodes which were sufficiently constant over the period of the experiment to be relied upon. They are intended to illustrate the usability of a new method of determining the hydrogen ion concentration of *living* tissues rath-

TABLE I.
pH of intestine of rat determined by the glass electrode.

Rat Fed Previously hr.	Duodenum Wall	Duodenum Contents	Ileum Wall	Ileum Contents	Cecum Wall	Cecum Contents	Colon Wall	Colon Contents
24	—	—	—	—	—	6.56	—	—
7	—	5.97	6.58	7.44	7.14	6.02	6.39†	—
24	6.36	—	7.44	7.50	7.10	7.51	6.39†	6.49†
24	—	—	—	—	6.10†	6.79	—	—
24	—	—	6.73†	6.69†	7.21	7.23	6.91	7.00
36	6.31	6.54	6.64	8.17	6.79	6.80	—	—
AVERAGE	6.34	6.26	6.85	7.45	6.87	6.82	6.56	6.75
(Live rats only)	—	—	(6.89)	(7.70)	(7.06)	—	(6.91)	(7.00)
Kofoid, McNeil, and Cailleau[3] (Q)	6.93	—	6.98	7.51	7.34	7.13	6.95	7.33
Redman, Willimott and Wokes[4] (Q) (C)	—	5.2	—	6.4	—	6.4	—	6.4
Abrahamson and Miller[5] (C)	—	5.83	—	5.88	—	—	—	—

[2] Kahler, H., and DeEds, F., *J. Am. Chem. Soc.*, 1931, **53**, 2998.

† Rat dead. (C) colorimetric method. (Q) quinhydrone electrode.

[3] Kofoid, C. A., McNeil, E., and Cailleau, R., *Univ. Calif. Publ. Zool.*, 1932, **36**, 347.

[4] Redman, T., Willimott, S. G., and Wokes, F., *Biochem. J.*, 1927, **21**, 589.

[5] Abrahamson, E. M., and Miller, E. G., PROC. SOC. EXP. BIOL. AND MED., 1925, **22**, 438.

er than to present an extensive series of pH determinations. The animal was alive during the course of readings except in those cases marked †; even here it had ceased breathing only 2-3 minutes before. Some results of other workers on hydrogen ion concentration of the digestive tract of the rat are appended for comparison.

7826 C

Tolerance of Fundulus Parvipinnis to Certain Bactericidal Substances.

CLAUDE E. ZOBELL AND NELSON A. WELLS.

From Scripps Institution of Oceanography, University of California, La Jolla, Calif.

The authors have described the bacteriology[1] and the pathology[2] of an infectious dermatitis of the Pacific killifish, *Fundulus parvipinnis*, and other marine fishes which has seriously handicapped the experimental work[3, 4] on these organisms. At certain periods during the last 3 or 4 years literally thousands of *Fundulus* have died of the disease in our laboratories and frequently important experiments have been terminated before results of value could be obtained. While the disease is known to occur in nature, probably the fishes are rendered more susceptible to infection by injuries sustained in the collecting nets and by subsequent handling. The elimination of those fishes which are visibly injured, the segregation of the infected, the disinfection of the tanks by chlorination, and the application of other ordinary precautions have been of little or no avail in controlling the epidemics. A hyperthermic treatment consisting of the gradual acclimatization of the fishes to water heated to 32°C. to 35°C. has been of prophylactic as well as of therapeutic value, but this procedure is slow and expensive. Therefore, it is desirable to find a more expedient method for the control of this and similar diseases.

Certain infectious diseases of fresh-water fishes[5] have been controlled by bathing the fish in solutions of selective germicides. With

[1] Wells, N. A., and ZoBell, C. E., *Proc. Nat. Acad. Sci.*, 1934, **20**, 123.

[2] ZoBell, C. E., and Wells, N. A., *J. Infect. Dis.*, 1934, **55**, 299.

[3] Wells, N. A., *Proc. Nat. Acad. Sci.*, 1932, **18**, 580.

[4] Wells, N. A., *Physiol. Zool.*, 1935, **8**, April.

[5] Plehn, M., *Praktikum der Fischkrankheiten*, E. Schweizerbart'sche, Stuttgart, 1924, 179.

this idea in mind experiments were designed to ascertain the tolerance of healthy fishes to such substances. Preliminary work with the inorganic salts of heavy metals removed this class of compounds from further consideration because, while large concentrations are tolerated by the fishes, the metals are precipitated from solution by the constituents of sea water, thereby decreasing their efficacy as germicides. Sumner[6] found that, due to their insolubility in sea water, certain salts of lead and arsenic in any proportion failed to kill *Fundulus heteroclitus* in 24 hours.

We used *Fundulus parvipinnis* of normal appearance and medium size (5 to 7 cm. in length). They were placed in groups of 2 or 3 in one liter of fresh aerated sea water (salinity ca 34.00‰) at a temperature of 15°C. to 16°C. Larger groups were not used because the vitiation of the water then became a factor which contributed to the distress and even the asphyxiation of the fishes. Different concentrations of various germicides were added to the water. Alcoholic solutions of some were used, but in no case was there enough alcohol to give more than 1%, and control experiments showed that double this amount of alcohol had no perceptible effect upon the fishes. Using new fishes in each case it was noted how long they could remain in the sea-water solutions of germicides before exhibiting signs of marked irritation, discomfort or distress; the maximum exposure they could tolerate and still survive upon being returned to running sea water; and the time required for their death

TABLE I.

Time of exposure to different concentrations of germicides in sea water which caused irritation, discomfort, or distress to Fundulus; their tolerance as indicated by longest exposure from which they recovered, and time required for their death.

Germicide	Concentration	Irritation	Tolerance	Death
Mercurochrome	1:100	Immediate	10 min.	35 min.
	1:1,000	15 min.	6 hr.	24 hr.
Acriflavine	1:1,000	45 "	30 min.	1 "
	1:10,000	None	4 hr.	20 "
Crystal violet	1:2,000	30 min.	20 min.	2 "
	1:10,000	—	4 hr.	20 "
Brilliant green	1:1,000	Immediate	?	5 min.
	1:10,000	"	4 min.	30 "
Acriviolet	1:1,000	20 min.	20 "	1 hr.
	1:10,000	None	7 hr.	20 "
Thymol	1:10,000	Immediate	3 min.	10 min.
Chloramine T	1:1,000	"	5 "	20 "
	1:2,000	"	20 "	35 "
Sodium perborate	1:100	"	10 "	30 "
	1:1,000	10 min.	6 hr.	24 hr.
Potassium permanganate	1:1,000	Immediate	2 min.	15 min.
	1:10,000	"	15 "	2 hr.

[6] Sumner, F. B., *Am. J. Physiol.*, 1907, **19**, 61.

in the solutions. This information is summarized in Table I and is based upon the average response of 4 or more fishes in each series.

The physical response of fishes being bathed in germicidal solutions is not a reliable criterion of their tolerance because there is little or no relationship between the apparent irritability and the toxicity of the germicides. In crystal violet the fishes exhibited no distress even after being in such solutions for a period of time which irreparably injured them, while in mercurochrome they were almost immediately distressed but quickly recovered, after surprisingly long exposures, upon transfer to fresh sea water. Acriviolet and acriflavine seemed to have a semi-narcotizing influence, fishes in these solutions remaining virtually motionless, and usually the first manifestation of distress was the loss of equilibrium in the pre-agonal stage.

With the exception of thymol and brilliant green, all of the substances in Table I were tolerated by *Fundulus* in concentrations which were potently germicidal towards the etiological agent of the infection, *Achromobacter ichthyodermis,* when suspended in sea water. Experiments with infected fishes revealed that crystal violet, mercurochrome and chloramine T were of no value in checking the infection when the latter was in an advanced stage, probably due to insufficient penetration. In fact, immersion of diseased fishes in germicidal solutions of these 3 substances to the limit of their tolerance seemed to aggravate the pathological processes by rendering the tissue more vulnerable to the invading microbes. Crystal violet and mercurochrome, as well as acriflavine and acriviolet, which appear to be least toxic and most bactericidal of any substances tried, may be useful in preventing the disease if used on healthy fishes shortly after they are exposed to the infection but before it has reached the pre-ulcerous stage of peripheral pallor. Sodium perborate, chloramine T and potassium permanganate are not recommended because of their relative instability in sea water and their pronounced irritability on the fishes.

In general, bathing these fishes in sea-water solutions of the germicides for a duration up to one-half the time reported under the column headed "Tolerance" in the table should provide for a generous margin of safety. The majority of fishes which have been so treated are still alive after several weeks. Also the repetition of this treatment at weekly intervals is tolerated, which shows that there are no cumulative injurious effects.

Missouri Section.

Washington University Medical School, January 9, 1935.

7827 P

Cornification of Vaginal Epithelium of Ovariectomized Rat Produced by Smearing.

NELSON J. WADE AND EDWARD A. DOISY.

From the Laboratories of Biology and Biological Chemistry, St. Louis University School of Medicine.

The histological examination of the vaginae of a number of spayed rats showing an unexpected response to the examination of the vagina by the smear method revealed that smearing alone, without the injection of an estrogenic hormone, may produce, under certain conditions, full cornification of the vaginal epithelium. The results of some of our experiments are summarized in Table I.

TABLE I.

No. animals	Smears per day	Rat units of theelin daily	Smear when killed	Cell layers in vaginal epithelium
13	none	none	none	2
14	1	none	—	3-6
6	2	none	±, ±±	4-8
35	3	none	±, ±±, ±±±, +	6-12
3	1	1	+	8-12
3	3	1	+	8-12

The spayed rats which were neither smeared nor injected presented a thin, smooth vaginal epithelium usually 2 cell layers in thickness with leucocytes migrating into the lumen. When the animals were smeared once, twice, and 3 times daily the typical section from the vagina showed a progressive thickening of the epithelium up to 12 or more layers with the usual desquamation of the surface cells, thus presenting a picture very similar to that obtained after the administration of estrogenic material.[1, 2]

[1] Allen, E., Sex and Internal Secretions, Chap. IX, 1932, Williams and Wilkins, Baltimore.

[2] Allen, E., Doisy, E. A., Francis, B. F., Gibson, H. V., Robertson, L. L., Colgate, C. E., Kountz, W. B., and Johnson, C. G., *Am. J. Anat.*, 1924, **34**, 133.

The vaginal smear picture[3] likewise underwent a progressive change when the animals were examined once, twice, and 3 times daily. The usual — smear observed when the animals were examined once daily changed to a ± or occasionally a ±± on and after the third day if the animals were examined twice daily. When the vaginal smears were made 3 times daily about 25% of the animals showed full + smears on the third or fourth day of treatment.*

In order to observe whether smearing 3 times daily would change the typical picture observed after the administration of theelin, 3 animals injected daily with one rat unit of theelin and smeared 3 times each day were compared with 3 other animals injected with the same amount of theelin but smeared once daily. No difference in response could be detected in sections of the vagina or in the vaginal smears.

Since a deficiency of vitamin A may cause cornification of the vaginal epithelium[4, 5] 8 rats which had been maintained on a high A diet from the time of weaning and 7 regular stock animals from the same source† were spayed and examined. These animals likewise showed cornified smears and the usual hyperplasia of the vaginal epithelium after being smeared 3 times daily, thus eliminating a deficiency of vitamin A as a factor.

The ultimate cause of the hyperplasia of the vaginal epithelium has not been determined but it is apparently produced by mechanical stimulation of the vagina either as a localized cellular reaction or as a response to nervous stimulation. Since irritation of the vaginal mucosa appears to be the chief cause of the hyperplasia, the method of making the vaginal smears in this laboratory may be mentioned briefly. A small amount of cotton wrapped about the end of a toothpick and moistened with water is inserted into the vaginal canal and gently rotated a few times, after which the accumulated material is spread on the slide, stained and examined. Although this procedure produces no gross injury to the vaginal mucosa it appears to be sufficient to stimulate the proliferation of cells in the vaginal epithelium. It does not, however, produce a generalized condition of estrus since no macroscopic changes were observed in the uterus.

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[3] Kahnt, L. C., and Doisy, E. A., *Endocrinology*, 1928, **12**, 760.

* Dr. S. A. Thayer will discuss the relation of these facts to the vaginal smear method of assay in a subsequent paper devoted to certain phases of bio-assay.

[4] Evans, H. M., *J. Biol. Chem.*, 1928, **77**, 651.

[5] Evans, H. M., and Bishop, K. S., *Anat. Rec.*, 1922, **23**, 17.

† These animals were secured from Purina Mills through the courtesy of Mr. H. C. Schaefer.

The age of the animal, within a range of 4 to 8 months, and the fact that the animal has or has not been used for assay of the estrogenic hormones apparently plays no rôle in this response to smearing. Likewise the reaction is not confined to our own strain of rats since the group of animals secured from an unrelated colony responded in the same manner. These same animals, as noted earlier, also eliminated a deficiency of vitamin A as a factor. The participation of the hypophysis may be eliminated also since a group of hypophysectomized rats showed a similar hyperplasia of the vaginal epithelium in response to smearing. This behavior is not peculiar to rats alone since a similar, though less extensive growth of the vaginal epithelium was observed in a group of mice smeared 3 times daily.

7828 P

Nuclear Inclusions Suggestive of Virus Action in Salivary Glands of the Monkey, Cebus fatuellus L.*

E. V. COWDRY AND GORDON H. SCOTT.

From the Anatomical Laboratory, Washington University, St. Louis, Mo.

Evidence is fast accumulating that we must recognize a special group of salivary gland viruses. All of them have been discovered by chance. They are so benign that attention was not directed to them by distinctive clinical symptoms. What attracted notice was the extraordinary hypertrophy of certain acinous, or duct, cells accompanied by the formation in their nuclei of inclusions resembling those caused by viruses.

The first inclusion-laden cells were reported under the heading of "protozoan-like bodies" in the parotids of 2 infants by Ribbert[1] and in the submaxillary glands of guinea pigs by Jackson.[2] Credit is due to Goodpasture and Talbot[3] for recognizing the close resemblance between the bodies in humans and guinea pigs and for pointing out the similarity of both to the intranuclear inclusions described by Tyzzer[4] in varicella. Lipschütz[5] then rediscovered the intranu-

*Aided by a grant from the Rockefeller Foundation for Research in virus disease.

1 Ribbert, H., *Centr. allg. Path. u. path. Anat.*, 1904, **15**, 945.

2 Jackson, L., *J. Infect. Dis.*, 1920, **26**, 347.

3 Goodpasture, E. W., and Talbot, F. B., *Am. J. Dis. Child.*, 1921, **21**, 415.

4 Tyzzer, E. E., *J. Med. Res.*, 1906, **14**, 361.

5 Lipschütz, B., *Arch. f. Dermat. u. Syph.*, 1921, **136**, 428.

clear inclusions in herpes admirably described and illustrated by Kopytowski,[6] and emphasized the great importance of these bodies in "inclusion diseases" in general. But Kuttner and Cole[7] and Kuttner[8] led in the demonstration that the inclusions in guinea pigs are actually caused by a virus. Investigators, while examining the salivary glands of other animals, have been on the lookout for nuclear inclusions with the result that they have been reported in rats,[9] moles,[10, 11] mice[12] and hamsters.[13] Finally Kuttner and Wang[13] have proved that the intranuclear inclusions in hamsters, mice and wild rats are caused by a virus which is very similar to the submaxillary gland virus of guinea pigs.

In the course of experiments on the effect of treatment with irradiated ergosterol we have also encountered large, nuclear inclusion containing cells in the parotids and submaxillaries of 2 adult *Cebus fatuellus* monkeys but not in the sublinguals. The presence of these bodies was not revealed by any particular clinical symptoms. The animals appeared to be quite normal when they were killed. The nuclear inclusions were only found in the intercalated and secretory ducts and not more than 4 were seen in any one section. Occasionally they were accompanied by a mild degree of lymphocytic infiltration. In their properties, the cells and the inclusions were remarkably uniform. No intermediate stages could be found between them and the neighboring unaltered duct cells. Final stages in disintegration of the hypertrophied cells were likewise lacking. Similar uniformity was emphasized by Farber and Wolbach[14] in humans and by the Rectors[10, 11] in moles. In shape the cells were roughly spherical with diameter ranging from 12-19.2 μ. The range in nuclear diameter was 8.4-13.2 μ and in inclusion diameter 3.6-7.2 μ. After fixation in formalin-Zenker and coloration with hematoxylin and eosin, the nuclear inclusions were strongly acidophilic. A distinct halo was always interposed between the inclusion and the nuclear membrane. Faintly basophilic cytoplasmic inclusions were often found between the nucleus and the lumen. These were roughly spherical and ranged in diameter from 1.5 to

[6] Kopytowski, W., *Arch. f. Dermat. u. Syph.*, 1900, **54**, 17; 1903, **68**, 55, 387.
[7] Kuttner, A. G., and Cole, R., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 537.
[8] Kuttner, A. G., *J. Exp. Med.*, 1927, **46**, 935.
[9] Thompson, M. J., *J. Infect. Dis.*, 1932, **50**, 162.
[10] Rector, L. E., and E. J., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 192.
[11] Rector, E. J. and L. E., *Am. J. Path.*, 1934, **10**, 629.
[12] Thompson, J., *Am. J. Path.*, 1934, **10**, 676.
[13] Kuttner, A. G., and Wang, S. H., *J. Exp. Med.*, 1934, **60**, 773.
[14] Farber, S., and Wolbach, S. B., *Am. J. Path.*, 1932, **8**, 123.

6 μ. The sublingual gland of 1, the parotids of 11 and the submaxillary glands of 13 *Macacus rhesus* monkeys given irradiated ergosterol in the same experiments did not show any inclusions like those in *Cebus fatuellus*. The status of these nuclear inclusions in *Cebus fatuellus* is on a par with that of those in humans and moles. They are sufficiently like the inclusions which have been demonstrated to be of virus etiology in guinea pigs to suggest a similar causation. The group of salivary gland viruses is probably a large one.

New York Meeting

New York Academy of Medicine, February 20, 1935.

7829 P

High Temperature Liver Death Syndrome.

JOHN E. SUTTON, JR. (Introduced by J. E. Sweet.)

From the Second Surgical (Cornell) Division, Bellevue Hospital, and the Departments of Surgical Research and Anatomy, Cornell University Medical College.

High temperature liver death has been an unexplained postoperative complication following cholecystectomy and bile duct surgery. Clinically there is an immediate postoperative rise in temperature. This elevation of temperature is rapid and progressive; reaching 107° to 109°F. (41.7°-42.8°C.), and death occurs in 48 hours or less. Postmortem examination of the liver shows passive congestion, disorganization of the cords of liver cells and widespread focal necrosis.

The nature of these lesions and the finding of hepatic arteries in close relation to the cystic duct in several recent operations upon the gall bladder suggested vascular damage as an etiological factor in this syndrome. To establish the possibility of such vessel injury at operation the hepatic vessels, the bile ducts and the gall bladders of 29 cadavers were dissected. In 16 of these 29 bodies the right hepatic artery was in such close relation to the cystic duct that it might easily have been injured during cholecystectomy.

Fourteen dogs were used to determine experimentally the clinical course and the liver changes following ligation of the hepatic arteries. Cholecystectomy was performed in all and at the same time dissections were carried out isolating the hepatic arteries. The largest artery was invariably ligated and in many, smaller vessels were also clamped. Six of the dogs showed immediate postoperative temperature rise to 104° to 105.8°F. (40° to 41°C.). Five of these animals died within 36 hours and one died during the fourth postoperative day. Examinations of their livers show diffuse nec-

rosis, disorganization of the liver cords and focal necrosis. These lesions are similar to those found in human livers after high temperature liver death. In the 8 dogs which did not die within 36 hours and which did not show typical elevation of temperature, injections of bismuth or starch mixtures demonstrated adequate collateral circulation to all lobes of the liver. The reaction to ligation of the hepatic arteries appears to be quantitative; if the liver is deprived of sufficient arterial blood the experimental animal develops the syndrome, high temperature liver death.

The essential lesion of this syndrome appears to be acute necrosis of the liver. Experimentally this lesion is produced by ligation of the hepatic arteries; clinically arterial occlusion may be due to thrombosis, embolism, or accidental ligation. On the basis of this work it is suggested that this postoperative complication be called acute postoperative necrosis of the liver.

7830

Decomposition of the Group A Substance in Horse Saliva by a Myxobacterium.

K. LANDSTEINER AND M. W. CHASE.

From the Laboratories of The Rockefeller Institute for Medical Research, New York.

The results of chemical investigation of preparations of the group A substance in horse saliva[1] which indicated that their activity may depend upon carbohydrate groupings suggested testing the effect upon the saliva substance, of a microorganism known to attack carbohydrates.

The discovery of a bacterium producing an enzyme which destroys the polysaccharide of Pneumococcus type III was made by Avery and Dubos.[2] Several other bacteria capable of splitting Pneumococcus polysaccharides have been found since by Sickles and Shaw.[3] While the action of the Avery and Dubos bacterium appeared to be specific, a Myxococcus which decomposes a variety of

[1] Landsteiner, K., *Science*, 1932, **76**, 351.

[2] Avery, O. T., and Dubos, R., *Science*, 1930, **72**, 151; *J. Exp. Med.*, 1931, **54**, 51.

[3] Sickles, G. M., and Shaw, M., *J. Inf. Dis.*, 1933, **53**, 38; PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 443; *J. Bact.*, 1934, **28**, 415.

bacterial polysaccharides[4, 5] was isolated from vegetable matter by Morgan and Thaysen.

The strain of Myxobacterium was sent to us through the kindness of Dr. Morgan. The culture was maintained on an aqueous extract of rabbit feces, 50 gm. to the liter, to which were added 0.05% $(NH_4)_2SO_4$ and 0.01% K_2HPO_4. The medium was used at pH 7.4 after Berkefeld filtration or in agar slants. The organism does not grow on most common nutrient media. The substance from horse saliva was dissolved in the fluid medium mentioned or in a simple mineral medium[2] in concentrations of 0.008% to 0.04%, and 3 cc. amounts were inoculated with 0.1 cc. of a 3 or 4 day fluid culture and kept at 37°C. The activity of the saliva preparation was determined by inhibition of the hemolysis of sheep cells by antisera prepared with human A blood.

In these experiments the substance was found to be destroyed during growth of the Myxococcus. Partial decomposition was noted after 2 days, and after a week only 2 to 10% of the original activity remained.

The activity of the saliva substance was not altered in actively growing broth cultures of a number of other microorganisms, such as *B. coli, St. aureus,* a strain of Proteus, *S. paratyphi-B.,* and *B. pyocyaneus.* Accordingly, the loss in serological activity of the saliva substance under the influence of the Myxobacterium tends to support the assumption of the carbohydrate nature of the specific structure.

Whether the action of the Myxococcus extends to the group A substances of human origin, which are serologically closely related to but may not be identical with that of horse saliva, remains to be determined.

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[4] Morgan, W. T. J., and Thaysen, A. C., *Nature,* 1933, **132**, 604.

[5] Favilli, G., and Biancalani, G., *Lo Sperimentale,* 1934, **88**, 337.

7831 P

Some Experimental Modifications of the Protoplasmic Surface.

W. J. V. OSTERHOUT AND S. E. HILL.

From the Laboratories of The Rockefeller Institute for Medical Research, New York City

Experiments on *Nitella* carried out by one or both of us show a variety of changes in the protoplasmic surface.

Placed in distilled water for 2 or 3 days cells of *Nitella* lose their ability to produce action currents, apparently because something, which may for convenience be called *R*, is dissolved out.*

Pinching the cell and thereby forcing sap into the surface can restore irritability as can an electric current passing from the sap into the surface.

What is said of irritability applies in general to the potassium effect,† but with some differences: distilled water can remove the potassium effect before irritability is lost and the potassium effect may then be restored by an action current.

The water in which cells have been standing contains substances which, when concentrated by appropriate methods, can restore irritability and the potassium effect, or the potassium effect alone, according to the method of concentration.

A number of solutions can restore irritability, or the potassium effect or both, *e. g.*, NH_3, NH_4Cl, tetraethyl ammonium chloride, guanidine, adrenaline, ephedrine, Solution A,‡ white of egg, milk, the blood of calves and of sheep, also human blood,** saliva, and urine.††

Where both effects are secured one is usually restored before the other and in some cases only one effect is obtainable. This indicates that irritability and the potassium effect are due to somewhat different causes.

Among the substances which have no restorative action are: urea, strychnine, brucine, codeine, veratrine, yohimbine, acetyl choline, aniline, toluidine, acetic acid, glycin, and guaiacol.

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* For experiments with a chloroform-water emulsion see Hill, S. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 413.

† *i. e.*, the large P. D. observed on leading off from 0.01 M KCl to 0.01 M NaCl. *Cf.*, Osterhout, W. J. V., *J. Gen. Physiol.*, 1930, **13**, 715.

‡ For the composition of this see Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933, **17**, 90.

** We employed the serum diluted 5 times with distilled water and containing enough oxalate to precipitate the calcium.

†† Used within 15 minutes after voiding.

7832 P

Influence of Ascorbic Acid of Diet on Sensitization of Guinea Pigs to Neoarsphenamine.

MARION B. SULZBERGER AND BERNARD L. OSER. (Introduced by Karl Landsteiner.)

From the Food Research Laboratories, New York.

Frei reported[1] that he was able to sensitize the skin of guinea pigs to neoarsphenamine. One of us (S) observed[2] a high degree of variation in the response of different series of guinea pigs to this type of sensitization. Mayer and Sulzberger[3] concluded that the composition of the diet was of fundamental significance, for, the animals receiving summer fodder were resistant to sensitization (only 0 to 12% becoming sensitized), whereas animals receiving winter fodder were sensitizable 75 to 100%. The "seasonal" difference in the diets depended upon the inclusion or omission of greens. Sensitization of guinea pigs to paraphenylene diamine as well as their reaction to infection with a virulent strain of tubercle bacilli have been reported to be similarly influenced by diet.[3, 4]

Our attention was directed to the vitamin C content of the rations. When pure crystalline vitamin C became available, it was decided to use the synthetic product* as the source of vitamin C instead of citrus or tomato juice.

Young guinea pigs weighing approximately 250 gm. were placed upon the scorbutogenic diet described by Demole,[5] consisting of 2 kilos oat flakes and one kilo dried whole milk (previously heated for 2 hours at 120°C.) made into cakes with the aid of 6 egg whites and baked on a greased pan for 20-25 minutes. 200 mg. per week of cod liver oil were fed each guinea pig and a small amount of dried hay supplied. After 10 to 15 days on this diet, the negative control animals commenced to lose weight and showed early signs of scurvy. Three to 4 weeks later they died and at autopsy further manifesta-

[1] Frei, W., *Klin. Wochschr.*, 1928, **7**, 1026.

[2] Sulzberger, M. B., *Klin. Wochschr.*, 1929, **6**, 253; *Arch. Dermat., and Syph.*, 1929, **20**, 669, 1930, **22**, 839; Sulzberger, M. B., and Simon, F. A., *J. Allergy*, 1934, **6**, 39.

[3] Mayer, R. L., and Sulzberger, M. B., *Arch. f. Dermat. u. Syph.*, 1931, **163**, 245; Sulzberger, M. B., and Mayer, R. L., *Arch. Dermat. and Syph.*, 1931, **24**, 537.

[4] v. Engel, P., *Arch. f. Dermat. u. Syph.*, 1933, **167**, 279.

* l-Ascorbic acid in the form of Redoxon manufactured by Hoffmann-LaRoche, Inc., to whom we are indebted for a liberal supply of the product.

[5] Demole, V., *Z. f. Vitaminforschung*, 1934, **3**, 89.

tations of scurvy were found. After the tenth day on this diet, the experimental animals were divided into 5 groups and fed individual daily supplements of ascorbic acid as follows: Group I, 0.25 mg.; Group II, 0.50 mg.; Group III, 0.75 mg.; Group IV, 1.50 mg.; and Group V, 2.00 mg. A fresh 1.5% solution of ascorbic acid was prepared each day by dissolving a weighed quantity of Redoxon in cooled, freshly boiled distilled water. This was administered orally by pipette. Demole and others reported that the minimum protective dose of ascorbic acid is about 0.6-0.7 mg. per day. Titration of the preparation used in each experiment by means of 2-6 dichlorophenol-indophenol indicated that the material was 100% ascorbic acid. All guinea pigs in our Groups I and II (receiving 0.50 mg. or less ascorbic acid) developed definite clinical signs of scurvy; whereas those in Groups III, IV and V (receiving 0.75 mg. or more) were practically completely protected. On the tenth day after they had begun to receive their daily supplements of ascorbic acid, the animals were injected intracutaneously in depilated areas of the right flank with 0.1 cc. of a 0.15% solution of neoarsphenamine (Metz) in sterile saline (0.15 mg. neoarsphenamine). Daily observations were made of the site of the injections. None of the animals showed an inflammatory response immediately following the injection or during the next few days. From the sixth to tenth day, the injection sites in a certain percentage of the animals "flared-up," *i. e.*, an erythematous and infiltrated papule became manifest, which in some instances reached the size of a bean and went on to central necrosis and scarring. On the twenty-eighth day, the animals were reinjected in the opposite flank with the same dose of neoarsphenamine. The reaction differed from the first in that sensitized animals showed an inflammatory reaction within 24 hours which increased in severity for a period of days. These manifestations were identical with those observed in the earlier experiments.

During the entire period of the sensitization experiment, the animals were kept on the same basal ration and the same daily supplements of ascorbic acid.

Table I records (a) intensity of the "flare-up" reaction at the site of the initial injection and the severity of the reaction following the second or test injection; and (b) the severity of the gross clinical signs of scurvy. These are correlated with the doses of ascorbic acid fed the various groups. The 6 animals that comprised the negative control group which died of acute scurvy, are not included in this table.

Distinct hypersensitivity was produced in the major number of

TABLE I.

Effect of varying doses of ascorbic acid on (a) hypersensitivity to neoarsphenamine and (b) protection against scurvy.

Group	Ascorbic Acid mg.	Guinea Pig No.	"Flare-up" at site of 1st injection	Response to Test Dose	Gross Signs of Scurvy
I	0.25	161	(+)	(+)	+++
		163	0	0	++++
		165	+	+++	++
		168	+	++	++
		169	+	(+)	+++
		170	++	†	++
		173	++	+++	++
		174	0	0	+++
		177	+	++	++
		182	+	+++	++
II	0.50	106	0	0	++
		135	(+)	+	++
		141	++	+++	++
		148	++	++	++
		150	0	(+)	+
		151	0	0	+++
		157	0	0	++
III	0.75	107	0	0	0
		108	++++	+++	0
		138	++	++	0
		142	+++	+++	+
		143	+++	+++	+
		149	+	++	+
		155	+	0	0
IV	1.50	109	++	++	0
		111	++	++	0
		140	++	+++	0
		144	0	+	+
		145	++	++	0
		146	0	(+)	0
		152	+	+++	0
		154	++	++	0
		156	++	++	0
V	2.0	160	0	0	0
		164	0	(+)	0
		166	0	0	0
		167	0	0	0
		171	0	(+)	0
		172	0	0	0
		176	0	0	0
		178	+	++	0
		179	0	(+)	0
		181	0	0	0

† Died 25 days after sensitizing dose.
(+) = minimal reaction. ++++ = Maximal reaction.

guinea pigs receiving 1.5 mg. or less of ascorbic acid per day, although only those animals that received 0.5 mg. or less were definitely scorbutic. On the other hand, in Group V, fed 2.0 mg. ascorbic

acid daily, only one animal showed a pronounced reaction; and of the remaining 9, 6 showed not the slightest evidence of sensitization. The animals in this group were completely protected from scurvy. It would appear, then, that the minimum dose which seems, in our experiments, to exercise an inhibitory influence upon the sensitization to neoarsphenamine, as shown by group V, is definitely higher than that which is sufficient to protect against gross scurvy.

Those animals in the less protected groups which failed to become sensitized were frequently precisely those which exhibited the most florid symptoms of scurvy and were cachectic. It is possible that their refractoriness to sensitization was based upon a different mechanism (cachectic energy?), *i. e.*, upon a mechanism which was not operative in Group V.

7833 P

Spectographic Determination of Lead in the Blood Serum.

I. B. WEXLER AND A. E. SOBEL. (Introduced by B. Kramer.)

From the Pediatric Research Laboratory, The Jewish Hospital of Brooklyn.

The chemical methods available for the quantitative determination of lead in blood, while accurate, require amounts of blood too large for routine clinical work. Spectroscopic methods are more sensitive and allow of the use of smaller amounts of material. They have, however, been employed for qualitative estimations only.* [1, 2, 3]

We have developed a spectroscopic method for determination of lead quantitatively which using a simple procedure can estimate from 0.0005-0.01 mg. of lead with an error of ±50%.

In principle the method consists of comparing either photometrically or visually, the intensity of lead lines from the unknown sample

* Since the completion of our work an article appeared on the "Quantitative Spectographic Determination of Lead in Urine," by Jacob Cholak, *J. Am. Chem. Soc.*, 1935, **57**, 104. The technique and apparatus used, however, is more involved and expensive than that described by us.

1 Rabinowitch, I. M., Dingwall, A., Mackay, F. H., *J. Biol. Chem.*, 1933, **103**, 707; *J. Biol. Chem.*, 1933, **103**, 725.

2 Shipley, P. G., Scott, T. F. McNair, and Blumberg, H., *Bull. Johns Hopkins Hosp.*, 1932, **51**, 327.

3 Blumberg, H., and Scott, T. F. McNair, *Bull. Johns Hopkins Hosp.*, 1935, **56**, 32.

with lines from admixtures of known amount of lead salt and the amount of sodium normally present in the quantity of serum used. The accuracy of the method is ±50% visually; this accuracy should be increased when using a photometer.

Since lead is a common contaminant, all glassware used was Pyrex which was steamed for 2-3 hours previous to use. The silica crucibles were similarly treated. Only lead-free needles and syringes were employed.

Spectroscope. The instrument used was the Hilger 4 E quartz spectroscope loaded with Eastman 33 plates. The spectrum produced by this instrument gives a long range of wave lengths extending well into the ultraviolet and permits the use of a large number of lines from which to draw conclusions.

Sodium Chloride Solution.—NaCl is recrystallized several times until free of lead. 8388 mg. is made up to 1000 cc. with redistilled water. 1 cc. of this solution contains 3.3 mg. of sodium.

Lead Chloride Solution—$PbCl_2$ is recrystallized several times. 13.4 mg. is dissolved with 1 cc. of lead-free redistilled nitric acid and then made to 1000 cc. 1 cc. of this solution contains 0.01 mg. lead.

Preparation of standards. To 1 cc. of NaCl solution in a silica crucible amounts varying from 0.1 cc. to 1.0 cc. of the $PbCl_2$ solution are added. The solutions are evaporated and the solid residue flashed. The concentration of lead in these mixtures corresponded from 0.1 mg. Pb^{++} to 1.0 mg. Pb^{++} in 100 cc. of solution. This was the range encountered in lead poisoning cases. The normal concentration of lead was not measured accurately. It is definitely below 0.1 mg. per 100 cc.

Treatment of the serum. 1 cc. of serum is placed in a silica crucible that is steamed for 2 hours previous to its use. The whole is dried in an electric oven at 110° for 3 hours and then ashed in a muffle furnace for 24-48 hours at 500°C. and then flashed.

Flashing. One set of H. S. Brand pure Acheson graphite electrodes 10 mm. in diameter and of convenient length are prepared for each determination. The electrodes are ground, the lower one for receiving the ash, flat—and the upper one to a fine point. They are supplied by a 110 volt direct current through a 4 amp. resistance. (See Fig. 1.)

The flashing is conducted in the following way: Roughly, one quarter of the crucible contents are placed on the electrodes with a platinum spatula, the electrodes are placed in juxtaposition, the current turned on, the electrodes are drawn apart and allowed to flash

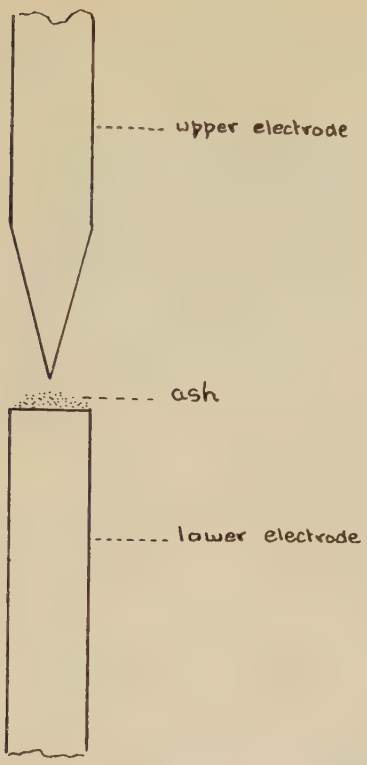

FIG. 1.
Diagram of electrodes and ash.

for 15 seconds. A second and third quarter are added in the same way. After the last portion has been added the flash is made to burn continuously for 10 minutes. (This time is found sufficient to volatilize the whole sample.)

The plates are then developed in Kodak D-61 for 12 minutes at 18°C., rinsed, fixed, washed and dried.

Determination of lead in serum. The concentration of lead in the serum is estimated by comparison of the intensity of lead lines against the standard spectra. An estimate of the concentration of lead is made by choosing those standard spectra whose lead lines are nearest in intensity to that of the unknown. In such manner the values are within ±50% when the comparison is made under a 25-fold magnification. The use of a photometer should increase the accuracy.

Results. Blood which showed no lead line on preliminary flashing gave satisfactory results on addition of known amounts of lead. Patients with clinical symptoms of lead poisoning showed concen-

trations in blood varying from less than 0.1 mg. to more than 1 mg. of lead per 100 cc. Normal values are definitely below 0.1 mg. per 100 cc. of serum.

TABLE I.
Sample Determinations of Lead in Human Blood Sera.
Values expressed as mg. Pb^{++}/100 cc. Serum.

Diagnosis	Subject	Lead Found
Lead Poisoning	Adult	.7
,, ,,	,,	.3
,, ,,	,,	.1
,, ,,	,,	1.0
,, ,,	,,	.2
,, ,,	,,	.5
Normal	Child	.03 appr.
,, + 0.5 mg. Pb in 100 cc.	,,	.5
,, + 0.3 mg. Pb in 100 cc.	,,	.3
,, + 0.2 mg. Pb in 100 cc.	,,	.2

7834 C

Demonstration of Hemorrhagins in Snake Venom by Means of the Chicken Embryo.

ERNST WITEBSKY, SAMUEL PECK, AND ERWIN NETER. (Introduced by Louis Gross.)

From the Laboratories of The Mount Sinai Hospital, New York City.

The venoms of various species of snakes differ in their content of toxic principles such as hemorrhagins, hemolysins and neurotoxins. While the hemolysins and neurotoxins can easily be determined, a reliable method for the demonstration of hemorrhagins has been lacking.

In the course of experiments with the chicken embryo it occurred to us that such a preparation might lend itself as a suitable test object for the study of hemorrhagins in venoms.

Fertilized chicken eggs are placed in an incubator at 42°C. for 3 days. The egg is opened preferably at the small pole; one-third or half of the shell is carefully removed and the egg poured into a beaker. Then, the beaker is covered with a watch glass and kept in the incubator at 37°-38°C. The body of the 3-day-old chicken embryo is surrounded by a vascular network of 3-4 cm. in diameter (Fig. 1).

A 1% solution of moccasin venom *(Ancistrodon piscivorus)* in physiological saline was prepared. Serial dilutions were then made up to 1.0 cc. with physiological saline. These were placed in the

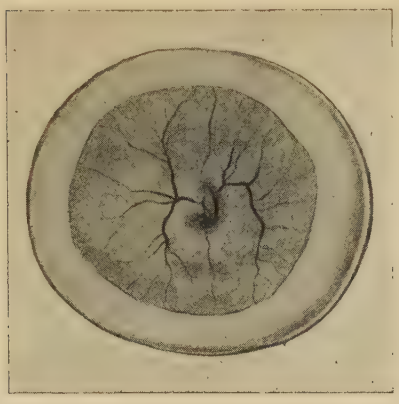

FIG. 1.

incubator at 37°C. for a few minutes and then added drop by drop to the chicken embryo. The embryo was returned to the incubator.

The addition of a sufficient concentration of moccasin venom to the chicken embryo causes petechial hemorrhages at various sites of the vascular network (Fig. 2). This phenomenon manifests itself in a few minutes. The hemorrhagic points rapidly increase in size and number (Fig. 3). Occasionally complete exsanguination of the

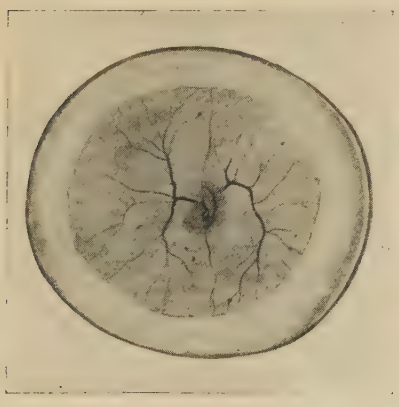

FIG. 2. 3 minutes.

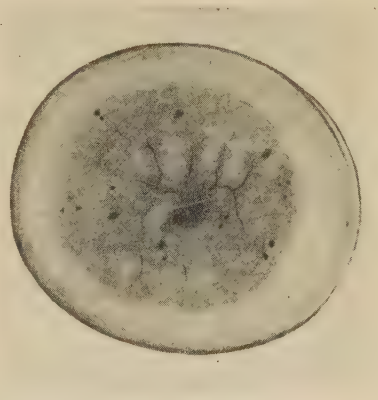

FIG. 3. 7 minutes.

embryo was observed from only one large hemorrhage. With the increase of hemorrhages the blood vessels lose their bright red color, their contours become less distinct and very soon the whole embryo assumes a dull glazed appearance which is followed by death.*

* Addition of one of the moccasin venoms (1%) caused a damage to the membrane of the egg yolk, so that its content ran out.

TABLE I.

The hemorrhagic effect of moccasin venom on the chicken embryo.

Venom I Amounts (Vol. 1.0 cc.)		Time in min. after addition of venom dilutions.														
	%	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
(1)	1	*	+	++	++	+++	+++	++	+++							
(2)	1/3	—	—	+	++	++	—	—	—	—	++	++	++	++	+++	—
(3)	1/9	—	—	—	—	—	—	—	—	—	—	++	++	++	++	—
(4)	1/27	—	—	—	—	—	—	—	—	—	++	++	++	++	++	—
(5)	1/81	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Venom II																
(1)	1	—	—	+	++		+++									
(2)	1/2	—	—	—	+		++	+++								
(3)	1/4	—	—	—	—		+	++	+++							
(4)	1/8	—	—	—	—		—	—	—	+	++	++	+++			
(5)	1/16	—	—	—	—		—	—	—	—	—	—	—	—	—	—

* — = no hemorrhage. + = appearance of the first petechiae.
++ = strong hemorrhage. +++ = death of the embryo.

Table I gives 2 illustrations of a series of 11 similar experiments. The data in the table represent observations on duplicate test objects. Physiologic saline in equal amounts, as well as a 1% solution of Bothrox atrox was added to the chicken embryos as control. Bothrox atrox did not give a purpuric reaction.

Furthermore, it can be seen from the table that there is a relationship between the concentration of the venom solution and the rapidity with which petechiae first appear. The latent period increases with the decrease of venom concentration. Once the hemorrhage is initiated, however, there is no striking difference to be observed as far as the degree and the number of hemorrhagic manifestations are concerned. Higher dilutions of the moccasin venom which just fail to produce hemorrhages in the chicken embryo sometimes caused bradycardia and death.

Summary. The addition of moccasin venom in sufficient concentration to 3-day-old chicken embryos produces hemorrhages in the vascular network. This method may be used for the demonstration of hemorrhagins in venoms. Further studies should be made to determine whether this method is applicable for the quantitative determination of hemorrhagins.

7835 C

Influence of Drugs on Chicken Embryo and on Its Reaction to Sera Containing Forssman Antibodies.

ERWIN NETER AND ERNST WITEBSKY. (Introduced by Louis Gross.)

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The addition of sera containing Forssman antibodies to 3-day-old chicken embryos which are taken out of the eggshell and kept in beakers, causes the following phenomenon: The vascular network contracts, the embryo turns around, sinks into the yolk and finally dies (Baumann and Witebsky[1]). This phenomenon is to be interpreted as the sequela of a reaction between the Forssman antigen of the chicken embryo and the corresponding antibodies of the added serum. It can be produced only by sera containing Forssman antibodies (normal "rabbit type" sera and Forssman antiserum). Thus, we are dealing with a phenomenon which parallels the so-called inverted anaphylactic shock of guinea pigs following the injection of Forssman antiserum.

The nature of the mechanism of anaphylactic phenomena is still a moot question. Some authors claim that they are due to physicochemical changes of the cells following antigen-antibody reaction;

[1] Baumann, A., and Witebsky, E., *Compt. rend Soc. Biol.*, 1934, **116**, 10; *Annales de l'Inst. Pasteur*, 1934, **53**, 282.

others believe that chemical substances formed by antigen-antibody reaction are the effective factor. The work of Dale, Kendall, Lewis, Manwaring and their coworkers has shown that histamine can produce reactions similar to or even identical with anaphylactic phenomena.[2] Since the addition of normal "rabbit-type" sera and Forssman antisera to the chicken embryo produces an anaphylactic reaction, it seemed of interest to determine whether histamine can produce a similar phenomenon in this preparation. However, various amounts of histamine (Imido-Roche) in the concentration of 1 mg. to 0.005 mg. dropped on the chicken embryo did not produce the phenomenon.* Thus, while there is a remarkable parallelism between the inverted anaphylactic shock of the guinea pig and the vascular phenomenon of the chicken embryo, there exists this marked difference in the susceptibility of this preparation to histamine.

Other drugs have been examined with respect to their ability to produce the vascular phenomenon in the chicken embryo (acetylcholine, atropine, epinephrine, ergotamine, strophanthin, digitalis, insulin, ether, chloroform). Although certain pharmacological effects were observed, none of the examined drugs caused the typical phenomenon.

The pharmacological effect of certain drugs toward the chicken embryo, however, is of interest since the 3-day-old embryo consists almost exclusively of vascular system in this stage of development. The first group of drugs tested comprised atropine, epinephrine, ergotamine and acetylcholine. These are known to affect the autonomic nervous system. They proved ineffective against the chicken embryo. Drugs of the digitalis group (digitalin, strophantine) caused a typical bradycardia, irregularity and stoppage of the heart beat.† As a third group, anesthetics were tried. The addition of a few drops of ether did not show any influence, while chloroform caused cardiac standstill in a few minutes.

There was next studied the influence of the drugs upon the production of the vascular phenomenon in the chicken embryo by means of "rabbit type" sera. None of the following drugs showed any inhibitory influence upon the production of the vascular phe-

[2] For literature and discussion see Dale, H., *Lancet*, 1929, **216**, 1285; Feldberg and Schiff, "Histamin," Berlin, Springer, 1930.

* The application of Witte-Peptone (1% solution in physiological saline) also failed to produce the vascular phenomenon in the chicken embryo.

† The toxic effect of strophanthin (ouabin in physiological saline) in a dosage of ½ mg. is not inhibited by previous or simultaneous addition of atropine sulphate. (0.5 mg.)

nomena—atropine, acetylcholine, ergotamine, epinephrine and histamine. Since the inhibitory influence of anesthetics on anaphylactic reactions is well known the study of their effect on the chicken embryo phenomenon was of interest. Ether in sublethal amounts does not impede the phenomenon. A few drops of 5% aqueous emulsion of chloroform produces a temporary cardiac standstill. Smaller amounts are ineffective and larger amounts cause death of the embryo. When an effective serum is added to a chicken embryo in which temporary cardiac standstill is induced by the addition of the proper amount (as determined by previous trial) of chloroform, the typical vascular phenomenon appears even though slightly retarded. This experiment shows that the added serum is absorbed, regardless of the arrest of circulation due to the cardiac standstill.‡ Too large an amount of chloroform, however, produces a permanent cardiac standstill. Such embryos do not react any longer to the addition of an effective serum.

A second form of temporary cardiac standsill, that caused by lower temperature, should be reported in this connection. If chicken embryos are placed in the icebox at a temperature of 4-6°C. a cardiac standstill results after a few minutes. When returned to the incubator the heart again starts beating. While the cardiac standstill due to chloroform does not prevent the phenomenon from occurring after the addition of effective sera, no reaction appears if the serum is added to an embryo whose heart beat is stopped under the influence of the cold. The phenomenon occurs, however, if the embryo is put back into the incubator. Apparently, therefore, either the serum is not absorbed in the cold or the suppression of the phenomenon is due to the fact that complement, which is necessary for the production of the vascular phenomenon of the chicken embryo, does not act at a lower temperature.

Summary. 1. In spite of the fact that histamine is able to cause reactions similar to anaphylactic phenomena in other animals, it does not produce in the chicken embryo the vascular phenomenon which follows the addition of Forssman antibody containing serum. 2. Drugs, such as acetylcholine, atropine, epinephrine, ergotamine, likewise fail to produce any reaction similar to the vascular phenomenon. 3. Drugs of the digitalis group induce bradycardia, irregularity and eventually produce cardiac standstill. 4. Chloroform

‡ The vascular phenomenon can also be produced in so-called monsters, *i. e.*, embryos with malformations consisting more or less of the vascular network only. Although there is no manifest circulation, the vessels of such monsters contract in the same way as in normal embryos. This observation was made 6 times.

stops the heart beat. With low concentrations of chloroform, a temporary cardiac standstill is produced. 5. Chicken embryos, treated with chloroform in a dosage which causes a temporary cardiac standstill react as normal embryos to the addition of effective serum, *i. e.*, they show typical vascular phenomenon. 6. The phenomenon cannot be produced in embryos whose heart beat is stopped by exposure to ice box temperature.

7836

Pulmonary Blood Velocity in Congestive Heart Failure. Velocity in Pulmonary Venous Circuit.

H. R. MILLER AND MATTHEW FURMAN. (Introduced by M. Ringer.)

From the Medical Service of Dr. L. Lichtwitz, Montefiore Hospital, New York.

Our observations demonstrate that there are changes chiefly, and sometimes solely, in the velocity of the pulmonary venous circulation clearly related to congestive heart failure. This velocity was estimated by utilizing 2 methods for studying pulmonary blood flow (1) the ether time[1] which serves as a measure of the rate of blood flow in the pulmonary arterial system and (2) the taste or saccharine time[2] which serves as a measure of the rate of blood flow through the combined arterial and venous circuit, the so-called "crude pulmonary circulation". By subtracting (1) from (2) we obtained readings of velocity in the pulmonary venous system. The normal average ether time is 5 to 9 seconds, the taste or saccharine time is 14 to 16 seconds, and the average velocity in the venous circuit is 6 to 9 seconds, practically identical with that in the arterial circuit.

Figures within normal limits were observed in 100 hospital individuals from various diseases, the greatest number of whom were free of cardiac disease and the rest recovered from congestive heart failure. On the other hand, in 30 hospital individuals with cardiac disease and in various stages of congestive failure lasting over many months, there were always prolonged saccharine time readings but the ether figures were within normal limits; the arithmetical

[1] Hitzig, W. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 935; Miller, H. R., *Ibid.*, 1934, **31**, 842.

[2] Fishberg, A. M., Hitzig, W. M., King, F. H., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 651

difference between the saccharine and ether readings was always greater than normal, in some instances between 36 and 52 seconds as against normal of 6 to 9 seconds. This evidence of marked retardation in the pulmonary venous channels was noted promptly upon the initiation of congestive failure and the retardation persisted until not only visible external signs but residual central pulmonary congestion disappeared completely. The sluggish blood flow in the venous limb of the pulmonary circulation in a number of instances was accompanied by ether figures which were also increased pointing to some degree of slowing in the arterial limb as well. These figures, however, of the arterial velocity seldom rose (from the normal 6 to 9 seconds) above 11 or 12 seconds and in very exceptional cases to 15 to 19 seconds. Such high ether readings were found in chronic cardiac diseases whether congestive failure was present or absent and led us to believe that disturbances in pulmonary arterial velocity were not the responsible factor. Instead we considered the possibility that alteration in the capillary and alveolar walls led to interference in the diffusion of gases. This problem is receiving further study.

Three further points belong in this report. (1) As a general rule, the extent and degree of congestive failure went hand in hand with the slowing of the pulmonary venous velocity and improvement in both was usually parallel; (2) Although the retardation of blood flow in the arterial channels was absolutely increased in some individuals with congestive failure, from a practical point of view this increase contributed only a minor fractional part to the slowing of the pulmonary circulation as a whole. (3) In the presence of myxedema following thyroidectomy for rheumatic heart disease, blood flow appeared to be markedly held up in the arterial and venous limbs despite marked amelioration from congestive failure.

7837 C

Maternal Behavior Induced in Virgin Rats by Prolactin.

OSCAR RIDDLE, ERNEST L. LAHR AND ROBERT W. BATES.

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Many observations of the past twenty years suggested that ovarian hormones activate or develop the maternal instinct in mammals. Wiesner and Sheard,[1] however, have recently given almost conclusive proof that those hormones are not capable of effecting an exhibition of maternal behavior in the rat; their studies also made it quite probable that in rats conditioned by previous "concaveation" the effective agent in the excitation of maternal behavior is some substance, or combination of substances, found in the anterior pituitary. The pituitary extracts used by Wiesner and Sheard were mixtures of the several anterior lobe hormones and though they could not identify the active agent or agents they state: "It seems indeed exceedingly unlikely that the same factor is responsible both for mammary secretion and maternal behavior."

Elsewhere we[2, 3] have shown that the broody instinct in laying fowl is produced by prolactin, and apparently by that anterior pituitary hormone only; neither estrin nor corpus luteum induces broodiness and care of young in fowl. Since the "incubation instinct" of lower vertebrates and the maternal instinct of mammals are probably phyletic equivalents the above-cited result strongly suggests that prolactin enters into the chain of reactions resulting in the exhibition of maternal behavior. The study of Wiesner and Sheard indicated that young virgin rats are particularly favorable animals on which to test the rôle of prolactin in maternal behavior.

We have used younger animals than were used by Wiesner and Sheard, have much reduced the period or amount of conditioning by concaveation, have sought (and found) maternal behavior while actively continuing injections, and have considered it advisable to arouse the rat ovaries to one full cycle of activity (estrin and progestin production) before attempting to induce maternal behavior. In this "priming" process we used Prolan (Elberfeld) on one-half

[1] Wiesner, B. P., and Sheard, N. M., Maternal behavior in the rat. Oliver and Boyd, London, 1933.

[2] Riddle, O., Bates, R. W., and Lahr, E. L., *Anat. Rec.* (Abstract), Supplement, 1934, **60**, 49.

[3] Riddle, O., Bates, R. W., and Lahr, E. L., *Am. J. Physiol.*, 1935, **111**, 352.

and follicle-stimulating hormone (F.S.H.) from anterior pituitaries of cattle on the other half of the test animals. The F.S.H. and prolactin used were made essentially by methods previously described.[4] The F.S.H. preparation (No. 267) contained some thyreotropic, luteinizing (and growth?) principles in addition, but was practically free from prolactin. The prolactin preparation used (No. 324) was free from F.S.H. (immature dove testis test) and thyreotropic (dove thyroid test) principles, and was heated to 60° for 5 hours (pH 8.0) to destroy growth hormone.

In our tests of behavior most of the technique used by Wiesner and Sheard was adopted. In all tests a young rat (1-7 days) was dropped into each cage daily and the behavior of the treated rat observed during 10 to 30 minutes. In a preliminary test 4 "unprimed" virgins 59 days old (and one multiparous rat isolated for 60 days) were injected for 5 days with 30 bird units daily of (unheated) prolactin; the multiparous rat received 60 units daily. At the end of the period of injection a 2-day-old young was left overnight (concaveation) with each of the treated rats of this series, and behavior was observed for 2 days after terminating the injections. No maternal behavior was observed in the case of any of these 5 rats. We thus obtained evidence that a quick response from "unprimed" virgins is probably not to be expected from single daily subcutaneous injections of prolactin; and also some evidence that a "spontaneous" occurrence of maternal behavior is not very frequent in our stock of virgin rats of this age. These preliminary tests are not further considered here. Our later tests involved priming with Prolan (or with F.S.H.), and in a short report Tietz[5] has suggested that urine of pregnancy may indirectly excite the nesting instinct in rabbits. If that is correct we assume that such dosage ultimately leads to a release of prolactin from the rabbit's pituitary.

Table I gives data obtained on 18 virgin rats, from 4 litters, aged 67-81 days at the beginning of treatment. They had been isolated from males since they were 30 days old; in 8 rats the vagina was closed and in 10 it was open. Nine were primed with Prolan (15 R.U. daily) for 5 days; 9 others were primed with F.S.H. (3 mg. daily) for 5 days. Thereafter, 10 rats were injected with prolactin, 15 units daily; 4 were used as a control without further injection; and 4 were treated with F.S.H. (+thyreotropic, etc.), 3 mg. daily. Two of the rats used (Nos. 222, 224) in Series I had been included

[4] Riddle, O., Bates, R. W., and Dykshorn, S. W., *Am. J. Physiol.*, 1933, **105**, 191.

[5] Tietz, E. B., *Science*, N. S., 1933, **78**, 316.

TABLE I.
Maternal Behavior in Virgin Rats after A.P. Hormones.

DAYS INJ. —— 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25		
SERIES I		
PROLAN CONTROL	222	N N NC ⊕ +
	235	
	231	C C C
	265	C
PROLAN PROLACT.	201	CNCN NC N N N N N N
	239	C C C N ⊕ ⊕ ⊕ ⊕ + + ⊕ ⊕ ⊕ ⊕ ⊕ ⊕
	236	N N C ○ N
	243	C + ⊕ ⊕ + ⊕ ⊕ ⊕ ⊕ + ⊕ ⊕ ⊕ ⊕ ⊕
	237	C C C
F.S.H. PROLACT.	210	C C C N
	263	C ⊕ ⊕ + ⊕ ⊕ + + ⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕
	232	C CN NC N ○ ○ ○ N N ○ ○ N ○
	268	C N ○ ○ N ○ N ⊕ ⊕ ⊕
	267	N NC ⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕
F.S.H F.S.H.	224	C CN C ⊕ + + ⊕ ⊕ + + + ⊕ + ⊕
	233	C N N?
	230	N C N + * + + + N N
	262	NC N N N N N N N
SERIES IA		
F.S.H. PROLACT.	230	⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕ + ⊕ ⊕
	233	+ + ⊕ ⊕ + ⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕
	262	N N N N N ⊕ N ⊕ ⊕ ⊕ +
PROLAN PROLACT	231	N N N N N +
	235	+ ○ ⊕ ⊕ ⊕ ⊕
	265	N ○ ○
PROLACT	222	+ ⊕ ⊕ ○ ○ ⊕ ⊕ ⊕
F.S.H WHOLE A.P.	201	N
	236	
	237	
PROLAN WHOLE A.P.	210	

⊕ RETRIEVES AND CUDDLES + RETRIEVES ONLY ○ CUDDLES ONLY
N NEST-BUILDING OVER YOUNG N NEST-BUILDING
C CONCAVEATED FOR 8-12 HOURS AT NIGHT
* Injected, by mistake, 14 hours earlier with prolactin.

in the preliminary series of prolactin injections; one was placed in the control group, the other in the F.S.H. group. It will be noted that these 2 rats—prolactin injected, and concaveated for 12 hours at 17-22 days previous to this later test—both gave exceptional results in their respective groups. We note that neither the nest-

building nor the care of young in nest (of course the rats can not suckle young) is the full equivalent of that usually observed in the multiparous rat.

Our experience indicates that the fullest expression of maternal behavior obtainable in these young virgin rats consists in the retrieving of young followed by cuddling or covering the young with the rat's own body. These two things may occur separately and are indicated by suitable symbols—separate or combined—in Table I. Nest-building, when the nesting material is placed directly over the young, seems definitely to indicate a true but lower level of development of the maternal instinct. In rat No. 201 this was the only expression of maternal behavior obtainable with prolactin; in rats 230, 237 and 210 even this grade of response was not obtained. These cases show that, with the small amount of concaveation employed and under other conditions of our test, some virgin rats do not show maternal behavior following prolactin administration. It is of course well known that some rats do not show such behavior after normal parturition, and there was a high percentage of failures with the mixed (or whole) pituitary extracts used by Wiesner and Sheard.

The important result shown by the tabulated data is that under prolactin the maternal instinct was developed in several cases. This development was of high degree in 5 of 10 tests. The fuller expression of the instinct appeared at 6 days after beginning prolactin dosage in 1 case, at 7 days in 1 case, at 8 days in 2 cases, and at 18 days in another. Retrieving or cuddling occurred between the sixth and tenth day in all of these 5 cases. In 2 additional cases cuddling occurred at 8 and 9 days, and in still another case significant nest-building was shown at 5 days. In 2 cases there was complete failure of prolactin to induce a recognizable aspect of maternal behavior. Of the 4 control rats 3 showed no maternal behavior; the fourth case, whose peculiar previous history has been noted, first showed some maternal behavior after 17 days of dosage. One of the 4 rats (No. 224) injected with F.S.H. had an earlier history of prolactin injection and showed maternal behavior on the 15th day of this F.S.H. dosage; another, No. 230, was accidentally injected once (at 14th day) with prolactin, and 14 hours afterwards began retrieving young; No. 233 was perhaps a total failure, and No. 262 began showing significant nest-building on the 17th day of F.S.H. injection.

The rats which responded poorly or not at all were retested (Series I A). After repriming their ovaries the control rats and

those of the F.S.H. group which gave no response (or only an attenuated response) while members of Series I, promptly gave a full or an increased response when later injected with prolactin. Similarly those rats of Series I which had failed to respond to prolactin dosage were reprimed and later injected with an *unfractionated* pituitary extract (10 mg. daily) whose prolactin content was at least 1 unit per mg. To the 13th day of such injection no rat that failed to respond to prolactin had responded to the administration of the several pituitary hormones.

Summary. In virgin rats aged 67-81 days, with ovaries stimulated by Prolan or F.S.H. administration during 5 days, the injection of purified and previously heated prolactin induced maternal behavior in 6 of 10 rats after 6-10 injections. Previous conditioning by concaveation was limited to a single 12-hour period in 4 of these rats. The maternal instinct, in somewhat varying degrees, was exhibited by all these rats while the daily subcutaneous injections were in progress. Six of 7 rats of the control and F.S.H. injected groups, all showing little or no maternal behavior during a 25-day test, later developed or greatly accentuated their maternal behavior after 1 to 8 days treatment with prolactin. Four rats which showed no maternal behavior under prolactin also later failed to show such behavior after repriming and 13 days of administration of unfractionated pituitary extract. Prolactin is the anterior pituitary hormone specifically concerned in the activation of maternal behavior in virgin rats.

7838 P

Effect of Dietary Fats on Growth and Composition of Tumors.

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While many studies of the effect of diet on tumor growth have been made, few deal with the quality of the dietary fat and its effect on the lipid content of the tumor. Sugiura and Benedict[1, 2] found that oral administration of 10% cod liver, olive, linseed and chaulmoogra oils had no influence upon susceptibility to and growth of

[1] Sugiura, K., and Benedict, S. R., *J. Cancer Research*, 1930, **14**, 311.
[2] Sugiura, K., and Benedict, S. R., *J. Cancer Research*, 1925, **9**, 204.

Flexner-Jobling rat carcinoma. Feeding 40% butter fat gave a decreased take and growth rate of Flexner-Jobling rat carcinoma, and increased regressions. The same diet had no influence upon the growth of a rat sarcoma. Freund, Lustig and Kellner[3] found that diet free from palmitin but rich in olein rendered half of a series of mice refractory to carcinoma inoculation, and caused a decreased carcinoma growth rate in the animals. Caspari[4] reported retarded growth of tumors in mice fed large amounts of butter fat or palmitin.

This report deals with the effect of a synthetic diet, varying only in the nature of the fat, on the growth of rat Carcino-Sarcoma No. 256 obtained from Dr. Frances Wood of Columbia University. The rats were inoculated subcutaneously in the groin by the trocar method. On the day of inoculation, they were placed on Diet 262, Sinclair.[5]

From 4 to 7 weeks after inoculation, the rat was decapitated, the tumor removed, weighed, and portions of the outside taken for analysis. Cholesterol, fatty acids, and phospholipids were determined by Bloor's method[6, 7] and the micro iodine numbers by Yasuda's modification[9] of the Rosenmund-Kuhnhenn[8] method.

The growth of the tumor is expressed by a formula of Bierich and Lang[10] involving the weight of the tumor in grams and its age in days from the time of inoculation, or $Wt = a \cdot t^2$; a (growth tendency of tumor) $= \frac{\text{weight}}{\text{time}} \times 10{,}000$. The growth tendencies of rat tumors on cocoanut oil diet (Iodine No. = 9) have been found to be larger than those of rat tumors on cod liver oil diet (Iodine No. = 157) (Table I).

In 4 experiments, Table I, the cocoanut and cod liver oil animals were inoculated with the same tumor and placed on the respective diets the day of inoculation. Similar results were obtained in one experiment in which menhaden oil, a fish body oil of Iodine No. 175, was substituted for cod liver oil.

Since cocoanut and cod liver oil differ so widely in iodine number it seemed best to compare the iodine numbers of the tumor phospholipid fatty acids. Table II shows results on cocoanut, men-

[3] Freund, E., Lustig, B., and Kellner, B., *Z. Krebsforsch.*, 1932, **37**, 355.

[4] Caspari, W., *Z. Krebsforsch.*, 1933, **38**, 355.

[5] Sinclair, R. G., *J. Biol. Chem.*, 1931, **92**, 245.

[6] Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

[7] Bloor, W. R., *J. Biol. Chem.*, 1929, **82**, 273.

[8] Rosenmund, K. W., and Kuhnhenn, W., *Z. Untersuch. Nahr.—Genussm*, 1923, **46**, 154.

[9] Yasuda, M., *J. Biol. Chem.*, 1931, **94**, 401.

[10] Bierich, R., and Lang, A., *Z. physiol. Chem.*, 1933, **216**, 217.

TABLE I.
Comparative Growth Tendency (a) of Rat Carcino-Sarcoma 256 on Cocoanut Oil and Cod Liver Oil Diets.

Experiment	1	2	3	4
Cocoanut Oil (I.N. 9)	108	97	114	144
	132	105	115	171
	138	121	139	202
	185	123	139	
	192		147	
			153	
			152	
			154	
			194	
Av. (21 animals) = 144	151	112	145	172
Cod Liver Oil (I.N. 157)	72	60	78	55
	86	86	107	104
	123	88	166	117
	135	92		163
		93		202
Av. (17 animals) = 107	104	84	117	128

haden and cod liver oil diets with a few on a fat-poor diet; Diet 3, Sinclair.[5]

TABLE II.
Influence of Food Fat on Degree of Unsaturation (Iodine Numbers) of Phospholipid Fatty Acids of Rat Carcino-Sarcoma No. 256.

Diet	Fat-Poor	Cocoanut Oil	Cod Liver Oil	Menhaden Oil
Determinations	6	17	18	8
Av.	84±2.3	94±3.1	106±4.8	112±2.1

Although Hartwell,[14] and Harris and Moore[15, 16] have shown that the incorporation of 12-15% cod liver oil in the diet inhibits the growth of rats, the retardation of tumor growth apparently is not due to such an effect. Sinclair (private communication to the author) has shown the growth curves of uninoculated rats on the cod liver oil diet used, to be essentially the same as those on the cocoanut oil diet, and normal Moreover, in the 21 rats on the cocoanut oil diet, the weights of the rats at death minus the weights of the tumors averaged 176 gm. while those of the 17 cod liver oil rats averaged 179 gm.

The inhibitory action of unsaturated fats on the growth of tumors can be explained by the theory that phospholipids function as oxygen transport agents within the cell.[11] The respiration of tumor cells is

[14] Hartwell, G. A., *Biochem. J.*, 1927, **21**, 1076.
[15] Harris, L. J., and Moore, T., *Biochem. J.*, 1928, **22**, 1461.
[16] Harris, L. J., and Moore, T., *Biochem. J.*, 1930, **23**, 1114.
[11] Sinclair, R. G., *Physiol. Rev.*, 1934, **14**, 351.

unusual. The incorporation of unsaturated fatty acids into the phospholipids of tumor cells may aid ordinary respiration as opposed to glycolysis, thus inhibiting tumor growth.

The iodine numbers of the phospholipid fatty acids of this tumor are low compared with those of muscle on the same diets. Sinclair[5] found for muscle: fat-poor diet—101; cocoanut oil diet—124; and cod liver oil diet—160; with menhaden oil probably in the range of cod liver oil values. There are several plausible explanations for these low iodine numbers. In the first place, the long chain unsaturated fatty acids of the diet may not enter the tumor which, being a carcino-sarcoma, consists of epithelium and connective tissue. Possibly connective tissue does not take up the unsaturated acids of the diet. A study of a pure carcinoma and sarcoma, comparing effects of these diets on similar rat tissues may settle this point.

In the second place, the ratio of saturated to unsaturated fatty acids in the tumor may be different from that in muscle. Determinations of amounts of solid and liquid fatty acids using the lead soap method, will clarify this point.

In the third place, the unsaturated fatty acids of the diet may enter the tumor phospholipids but be immediately reduced or saturated. Boyland[12] and Harris[13] have shown that tumors contain some as yet unidentified reducing substance in addition to glutathione and ascorbic acid. It is not known whether this substance reduces unsaturated fatty acids.

7839 C

Reaction to Differentiate Vitamin A from Carotene by Means of Antimony Trichloride.*

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The Carr-Price reaction for vitamin A and carotene consists in the development in chloroform solution of a characteristic blue color

[12] Boyland, E., *Biochem. J.*, 1933, **27**, 802.

[13] Harris, L. J., *Nature*, 1933, **132**, 605.

* The carotene used in our work was obtained from the S.M.A. Corporation of Cleveland, Ohio, and consists largely of β-carotene with small amounts of α-carotene.

with antimony trichloride.[1] Recently Rosenthal and Erdelyi[2] have described a test in which antimony trichloride in the presence of pyrocatechin serves to distinguish vitamin A from carotene. A blue color is formed at room temperature in a chloroform mixture made up to a total volume of 5 cc., containing a solution of an oil rich in vitamin A, 2 cc. of antimony trichloride reagent, and 1 cc. of 0.5% pyrocatechin in chloroform. Immediately after mixing, the mixture is transferred to a water bath and maintained at a temperature of 60°C. for 1 to 2 minutes. During the treatment with heat the original blue color changes to a violet-red. These investigators report the violet-red color to be more stable than the original blue of the Carr-Price reaction. Under similar treatment a reaction mixture containing carotene yields a blue color which does not change on heating. Recently Rosenthal and Erdelyi[3] have reported that in addition to pyrocatechin, other polyphenols or their derivatives, such as hydroquinone, veratrole, and guaiacol, may be employed.

To make the studies of the Rosenthal-Erdelyi reaction, we made use of halibut liver oil assayed to contain 50,000 U.S.P. units of vitamin A per gram. A solution in chloroform was made of such a concentration that 1 cc. was equivalent to 100 units of the vitamin. Carotene solutions in chloroform were made up fresh in concentrations of 0.3 mg. of the pigment per cc. Antimony trichloride reagent was prepared by washing a new sample of antimony trichloride with chloroform and adding 30 gm. of the washed compound to 100 cc. of the chloroform. Two solutions of 0.5% pyrocatechin in chloroform were used. One was a freshly made solution, and the other prepared and aged for 21 days in an ordinary clear glass-stoppered bottle exposed to daylight but not to direct sunlight. The chloroform used for all solutions and employed in all manipulations was the U.S.P. grade containing less than 1% of ethyl alcohol.

Our studies indicate that the use of antimony trichloride and heat is all that is needed to carry out a differential test for vitamin A and carotene. We recommend that 2 cc. of the antimony trichloride reagent be added to about 3 cc. of a chloroform solution of the vitamin A-rich material, or to a solution of carotene. The reaction mixture should be allowed to stand for one minute to permit the blue color to develop to its full intensity before heating at 60°C.

[1] Carr, F., and Price, E. A., *Biochem. J.*, 1926, **20**, 497.

[2] Rosenthal, E., and Erdelyi, J., *Biochem. Z.*, 1933, **267**, 119; *Biochem. J.*, 1934, **28**, 41.

[3] Rosenthal, E., and Erdelyi, J., *Biochem. Z.*, 1934, **271**, 414.

on the water bath for 2 minutes. As a result of heating the blue color at first formed in the reaction between vitamin A and the antimony trichloride changed to pink, rose, red tinted with violet, or a deep wine-red depending upon the quantity of vitamin A present. A rose color developed with 0.03 cc. to 0.1 cc. of a chloroform solution of halibut liver oil (3 to 10 units of vitamin A).

TABLE I.
Antimony Trichloride and Halibut Liver Oil.

Halibut liver oil solution—1 cc. is equivalent to 100 U.S.P units of Vitamin A	Chloroform	0.5% Pyrocatechin, fresh	0.5% Pyrocatechin, old	Antimony trichloride solution	Color before heating	Color after heating	Color on standing 30 min. after heating
cc.	cc.	cc.	cc.	cc.			
0.00	2.00	1	0	2	none	none	none
0.00	2.00	0	1	2	"	"	"
0.03	1.97	1	0	2	faint blue tint	"	"
0.03	1.97	0	1	2	none	"	"
0.03	2.97	0	0	2	faint blue tint	faint pink tint	faint pink tint
0.10	1.90	1	0	2	blue	pink	pink tint
0.10	1.90	0	1	2	faint blue	none	none
0.10	2.90	0	0	2	blue	rose	rose
1.00	1.00	1	0	2	"	red	"
1.00	1.00	0	1	2	"	rose tint	pink
1.00	2.00	0	0	2	"	wine-red	wine-red

A red-violet color formed with 0.5 cc. of a chloroform solution of halibut liver oil, an amount equivalent to 50 units of vitamin A. A wine-red color appeared with 1 cc. of a solution of the oil (100 units of vitamin A). In the absence of pyrocatechin the color developed contained more red and less violet.

Carotene yields a blue color with antimony trichloride. The blue color persists, however, after heating. A carotene solution containing 0.3 mg. of the pigment gave a greenish blue tint changing immediately to blue and remaining unaltered on the application of heat. The interaction of carotene and antimony trichloride yields the full blue color more rapidly in the absence of pyrocatechin.

Our work indicates that the presence of pyrocatechin, as suggested by Rosenthal and Erdelyi, is unnecessary. In fact, pyrocatechin was found to inhibit the development of the red color with vitamin A during the treatment with heat, and also to inhibit the formation of the blue color with carotene at room temperature. We observed that the intensity of the red color obtained in a heated

reaction mixture containing antimony trichloride, pyrocatechin and halibut liver oil was less than the intensity of the color obtained in a similar mixture with the pyrocatechin omitted. The difference in color intensity was noted over a wide range of vitamin A content, from 10 to 200 units. Rosenthal and Erdelyi[3] have also recorded results which indicate that phenols other than pyrocatechin exert an inhibitory effect. They reported a scarcely discernible blue color when antimony trichloride reacted with a solution containing 0.001 mg. of vitamin A per cc., but upon addition of guaiacol and the application of heat the color disappeared. We have been able to obtain a blue color, which changed to rose on heating in the presence of a small quantity of halibut liver oil equivalent to 3 units of vitamin A. A similar quantity of halibut liver oil treated with antimony trichloride and pyrocatechin gave a blue color in the cold, but no pink or rose on heating.

The aged pyrocatechin solution exerted an even greater inhibitory effect than the fresh chloroform solution of the phenol. With 0.03 cc. of the halibut liver oil solution (equivalent to 3 units of vitamin A), 1 cc. of the fresh pyrocatechin solution and 2 cc. of the antimony trichloride solution, a faint blue tint developed at room temperature. When this mixture was heated the blue tint disappeared without further development of color. With a mixture using the same quantity of vitamin and 1 cc. of the old pyrocatechin solution, no color appeared before or after heating. With 0.4 cc. of halibut liver oil solution equivalent to 40 units of vitamin A, a blue color formed in the presence of 1 cc. of old pyrocatechin solution. The color disappeared on heating without the subsequent formation of a red-violet color. In a control experiment in which fresh pyrocatechin was used the blue color appeared before heating, and the red-violet color after heating.

Cod liver oil gave similar responses to haliver oil. Almond oil, olive oil, linseed oil and castor oil gave atypical reactions.

Summary. Antimony trichloride may be used effectively as a reagent to differentiate vitamin A from carotene. In chloroform solution antimony trichloride and carotene interact with the formation of a blue color, which persists after heating on the water-bath at 60°C. Under similar conditions antimony trichloride and vitamin A-bearing oils develop at room temperature a blue color. On the application of heat the blue color changes to rose, violet-red or wine-red, depending upon the concentration of the vitamin.

Pyrocatechin, which Rosenthal and Erdelyi employed along with the antimony trichloride reagent to differentiate vitamin A from

carotene, is not only needless, but it actually inhibits the formation of the blue color with carotene and of the blue color with oils rich in vitamin A at room temperature and of the rose or violet-red after heating. Aged solutions of pyrocatechol exert even greater inhibitory powers than fresh solutions.

7840 P

Cultivation of the Johne's Bacillus in a Synthetic Medium.*

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Twort and Ingram[1] succeeded in isolating the causative organism from the intestine of a cow which had died of Johne's disease, by using a special medium containing the dead cells of tubercle bacilli. They later found that other acid-fast organisms would give the same stimulation, the timothy grass bacillus, *Mycobacterium phlei,* giving especially good results. Since then the cells, various extracts of the cells, or the products of growth of *M. phlei* have been incorporated in the media used to cultivate the Johne's bacillus. The nature of the "essential substance", which may be chemical or physico-chemical has, however, remained obscure.

Johne's disease (paratuberculosis) is a chronic enteritis affecting the cow and the sheep and caused by the multiplication of the Johne's bacillus in the intestinal mucosa, the impairment of the function of which results in the emaciation of the animal. The disease is usually fatal. The disease occurs in isolated herds in the United States but is not yet of serious economic importance. In France, however, the disease has spread within the last 20 years from 2 to 41 departments of the country according to Rinjard.[2] It would seem, therefore, that the United States would profit by combating the disease before it becomes more widely distributed. In the eradication we are hampered by the lack of a sufficiently good diagnostic agent. A "Johnin" prepared like old tuberculin neces-

* Published with the consent of the Director of the Agricultural Experiment Station. Aided by a grant from the special research funds of the University.

[1] Twort, F. W., and Ingram, G. L. Y., A Monograph on Johne's Disease. Baillière, Tindall and Cox, London, 1913.

[2] Rinjard, M. P., *La paratuberculose bovine en France.* Office International des Epizooties R 51, 1934.

sarily contains fractions of *M. phlei,* which Dunkin[3] reports may lead to a false positive reaction, and the potency of different batches varies widely and cannot be easily standardized. For these reasons avian tuberculin has been used to diagnose Johne's disease by Hagan and Zeissig[4] and others. It would seem, however, that a specific product should yield better results than the avian tuberculin.

We have found that under certain conditions the Johne's bacillus can be induced to grow in Dorset's[5] synthetic medium without the addition of any material of unknown composition such as the cells of *M. phlei.* We have successfully carried one of our strains of the Johne's bacillus through 10 subcultures on the synthetic medium during the last 2 years. The growth of the Johne's bacillus on media not containing the "essential substance" in one subculture has been reported by Dunkin.[3] Such evidence is not conclusive of growth without the "essential substance", since some of it might have been carried over with the inoculum to the first subculture.

The conditions necessary for the growth of the Johne's bacillus on the synthetic medium are that the thin, even-surfaced rather than the thick, granular type of pellicle growth shall be used as the inoculum, and that a mass of growth at least 7 or 8 mm. in diameter per 50 cc. of medium shall be used. The inocula are floated on the surface of the medium.

The type of growth on the synthetic medium differs from that on the same synthetic medium containing in addition the dead cells of *M. phlei.* On the latter medium the growth spreads in a thin, coherent film over the surface in 3 to 4 weeks and then thickens and wrinkles, attaining the maximum in 2 months; whereas on the synthetic medium the growth occurs in discrete particles, thin in some places and thick in others. The total amount of growth on the synthetic medium is never as great as that on the phlei medium. At the end of 5 months the growth on the phlei medium has retained the same appearance as it had after 2 months, while the growth on the synthetic medium is wet and sunken. As good growth occurred in the tenth subculture as in the first subculture on the synthetic medium. If transfers are made from any subculture back to the phlei medium, the type of growth characteristic of the phlei medium at once appears.

The cultivation of the Johne's bacillus on a synthetic medium

[3] Dunkin, G. W., *J. Comp. Path. and Ther.,* 1933, **46**, 159.

[4] Hagan, W. A., and Zeissig, A., *Ann. Rpt. Cornell State Veterinary College,* 1927-28, 172.

[5] Henley, R. R., *Am. Rev. Tuberc.,* 1929, **19**, 660.

has enabled us to prepare a solution of the Johne's bacillus protein for diagnostic purposes according to the ultrafiltration method of Seibert[6] for preparing tuberculin. This "Johnin" eliminates certain disadvantages of previous products, since it contains none of the foreign proteins which are introduced by the use of the phlei medium, and is practically free of the crystalloids of the culture medium. It can be easily standardized by measuring the amount of protein precipitated from a sample of the solution by trichloracetic acid.

Conclusions. The dead cells of *M. phlei* contain an *accessory* rather than an *essential* growth substance for the Johne's bacillus.

A purified "Johnin" containing no proteins other than the Johne's bacillus proteins has been manufactured for use as a diagnostic agent.

7841 P

Effect of Adrenalectomy and Hypophysectomy upon Experimental Diabetes in the Cat.

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From the George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia.

We have previously reported some preliminary observations upon 5 adrenalectomized-depancreatized cats[1] and upon 2 hypophysectomized-depancreatized cats.[2] In the first series of adrenalectomized-depancreatized animals the operations were performed in 3 stages, but in the present group of 5 animals we have found it possible to remove the remaining adrenal and all the pancreas at one operation. These animals have survived 8, 9, 9, 16 and 28 days respectively. By this procedure we have avoided any loss of weight prior to total adrenalectomy and pancreatectomy, and since the results obtained are substantially identical with the previous ones the criticism of Ring[3] that previous inanition was responsible for the effects observed would appear to be unfounded. In addition, depancreatized

[6] Seibert, F. B., *J. Biol. Chem.*, 1928, **78**, 345.

[1] Long, C. N. H., and Lukens, F. D. W. *Science*, 1934, **79**, 569.

[2] Long, C. N. H., and Lukens, F. D. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 326.

[3] Ring, G. C., *Science*, 1934, **80**, 97.

cats treated with insulin or cats in whom a pancreatic remnant is left lose considerable weight, but when insulin is withdrawn or the last piece of pancreas extirpated, they do not survive any longer than animals totally depancreatized in one stage.

In the present series of experiments we have compared the urinary glucose, nitrogen and acetone bodies of 3 normal, 4 depancreatized, 4 hypophysectomized-depancreatized and 5 adrenalectomized-depancreatized cats. All these animals were fasting. In the case of the depancreatized cats the observations were made during the remainder of life and in the other animals for 5-7 days from the day of operation. The results are in Table I.

TABLE I.

Operation	Survival (days)	Urinary glucose gm./kilo/day	Urinary nitrogen gm./kilo/day	Urinary acetone bodies mg./kilo/day	Fasting blood sugar mg. %
Normal	—	0	0.5 (0.4-0.8)	11 (1-19)	—
Depancreatized	4 (2-5)	3.9 (2.4-5.5)	1.6 (1.2-2.4)	148 (40-370)	346 (226-788)
Adrenalectomized and Depancreatized	16 (8-28)	0.4 (0.0-1.2)	0.6 (0.2-1.0)	7 (2-13)	218 (80-342)
Hypophysectomized and Depancreatized	39 (18-85)	1.7 (1.2-2.6)	1.0 (0.8-1.2)	7 (2-11)	270 (129-291)

It will be observed that the well known high excretion of glucose and nitrogen is much reduced in the doubly operated animals while the characteristic ketonuria is almost entirely absent. The latter does not become evident throughout life unless steps are taken to induce it. Rietti[4] has reported comparable finding in the hypophysectomized-depancreatized dog, but so far as we know such observations have not been previously made in adrenalectomized-depancreatized animals.

It would appear that the continued survival of the doubly operated animals is in large measure due to the absence of ketosis (and acidosis) and in the case of the adrenalectomized-depancreatized cats can be prolonged for a period equal to that of hypophysectomized-depancreatized cats provided ample amounts of cortical extract are administered.

Thus one of our adrenalectomized-depancreatized cats was maintained for 28 days and then killed in good health. It received 5-10 cc. of "Eschatin" daily. At the time of death the CO_2 combining power was 40 volumes %, the liver glycogen 1% and the liver fatty

[4] Rietti, C. T., *J. Physiol.*, 1932, **77**, 92.

acids 17%. In this animal we have observed some indications that the degree of glycosuria is proportional to the adequacy of the cortical hormone therapy of Hartman and Brownell.[5]

We have also carried out a number of observations on the effect of anterior pituitary extracts and epinephrine upon the ketonuria and glycosuria of these doubly operated animals which will be reported at a later date.

7842 P

Further Experimental Lesions of the Pyramidal Tracts.*

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It has been shown (Marshall[1]) that a lesion of the pyramidal tracts in the medulla produces on the whole a less severe disorder of motility than does removal of the motor cortex. Evidence has also been presented which would suggest that lesions of the rubrospinal tracts interfere with 2 groups of reflexes, the "Berührungsreflexe" of Munk and the contact placing reactions of Rademaker, which are dependent upon the integrity of the motor cortex. The conclusions were drawn that the pyramidal tracts do not form the only significant pathways of discharge from the motor cortex, and that other "extrapyramidal" tracts, particularly the rubro-spinal, participate as well.

If these conclusions are correct, then the removal of the motor cortex subsequent to a section of the pyramidal tracts should produce a paralysis, the severity of which might be more or less proportional to the functional importance of the extrapyramidal pathways descending from the motor area. The present experiments attempt to determine this. In 2 cats the left pyramid was sectioned, and 10 months was allowed for the degeneration of the pyramidal system and its cells of origin in the cerebral cortex. The motor area for the limb muscles (area 4 of Brodmann) which includes the

[5] Hartman, F. A., and Brownell, K. A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 834.

*Aided by a grant from the Rockefeller Foundation Fluid Research Fund of Yale University School of Medicine.

[1] Marshall, C., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 68; *Am. J. Physiol.*, 1934, **109**, 178; *Arch. Neurol. and Psychiat.*, 1934, **32**, 778.

entire post-cruciate gyrus and the lateral half of the pre-cruciate gyrus was then removed on the left side. On the 16th and 17th day the animals were sacrificed and the brain and spinal cord removed. The gross preparations showed the lesions to be as described. Marchi and Pal-Weigert studies are in progress.

The symptoms following the first operation were similar to those previously reported. There were a number of initial symptoms which gradually disappeared, so that prior to the second operation the defects were hardly noticeable on casual observation. On closer inspection there could be seen a slight defect in the gait of the right hind leg, and occasional slight abnormalities of posture in the same limb. There was no definite extensor hypertonus but when the animal was held off the ground the right hind leg took a more extended position than the left and it was used less in struggling. The "Berührungsreflexe" and the contact placing reactions were underactive in both legs on the affected side, and the capacity to walk along a narrow track was poor.

Following the second operation the animals were again paralyzed to a degree not much less than following the first operation. The anesthetic used was sodium amytal and the animals slept through the day of the operation. On the following day one cat was able to walk with some staggering while the other did not progress until the day after. The capacity to walk improved rapidly, although at the end of the period of observation there was a slight stiffness and an occasional scraping of the toes of the hind leg on the affected side.

The defects in posture also reverted to the condition they were in following the first operation; there were marked spontaneous abnormalities and a failure to correct abnormal postures imposed upon the limbs. As before there was a gradual recovery, and at the end only slight defects were apparent, such as an undue twisting of the pivoting leg on a turn.

The "Berührungsreflexe" of Munk and the contact placing reactions of Rademaker showed no recovery following the second operation. These have previously been shown to be permanently abolished following extirpation of the motor cortex. The visual placing reactions were not abolished but were somewhat reduced on the affected side. The hopping reactions were markedly affected initially but showed considerable improvement with time. The capacity to walk along the rungs of a narrow ladder was seriously and permanently disordered.

The resistance to passive flexion (extensor hypertonus) was

moderately increased on the affected side when the animal was held in certain positions, although it was not enough to interfere seriously with the gait. The degree of hypertonus diminished with time, although the leg still tended to assume an extended posture when its normal fellow would be flexed. The normal active resistance to passive extension was much reduced, and struggling was much less on the affected side.

The results would appear to confirm the thesis that the "extrapyramidal" descending pathways from the motor cortex form an important functional system in the cat.

7843 C

The Reactions of Rat and Mouse Eggs to Hydrogen Ions.

B. VINCENT HALL. (Introduced by J. S. Nicholas.)

From Osborn Zoological Laboratory, Yale University.

The mammalian egg is enclosed by a transparent membrane, the oölemma or zona pellucida, which disappears as the blastocyst implants itself in the uterine wall. Various suggestions, unsupported by experimental evidence, have been advanced as to the factors concerned in the removal of the egg membrane within the uterus. In the course of studies *in vitro* on rat and mouse eggs, it was found that eggs placed in acidified Ringer's solution rapidly lost the zona. No difference was observed between the reactions of rat and mouse eggs but occasionally eggs from the same animal showed marked differences. In one case, the zona of a 4-celled rat egg swelled and disappeared in 10 minutes, while the zona of a second egg (8-celled) remained unaffected after several hours in the same drop of fluid (pH 4.3). Practical use of this method of removing the zonae has been made in the study of the development of the isolated blastomeres of the rat.[1]

Rat and mouse eggs, from the 2-celled to early blastocyst stages, were cultured at room temperature by the hanging drop method in depression slides. For aid in these experiments, I am greatly indebted to Dr. J. S. Nicholas, who removed the uterine tubes from the animals and obtained most of the eggs. The eggs were obtained by mincing the uterine tubes in unbuffered Ringer's fluid in an embryological watch glass, and after a few minutes removing the

[1] Nicholas, J. S., and Hall, B. V., *Anat. Rec.*, **58**, 83.

eggs with a capillary pipette under a binocular dissecting microscope. The eggs were transferred to a second watch glass filled with unbuffered Ringer's fluid and then to the test solutions.

Test solutions were prepared by diluting unbuffered Ringer's fluid, made up to 0.001 *M* HCl, with non-acidified Ringer's solution to the concentration desired. Hydrogen ion concentrations were checked electrometrically by the quinhydrone electrode. Calcium-free Ringer's fluids were used because experience proved that the tendency of the naked blastomeres to adhere to the glass walls of the dishes and pipettes is less in the absence of the calcium ion. Experiments were also conducted with unbuffered Ringer's solutions made alkaline with NaOH. The zonae are not visibly affected in 0.001 *N* NaOH, but in the alkaline solutions, the blastomeres swell and become transparent.

The action of the acidified Ringer's fluid is first noticeable in the swelling of the oölemma, which frequently increases to nearly double its normal thickness. The smooth outer contour of the zona changes to a wavy outline and may become fringe-like. These changes in the zona, observed *in vitro,* appear to be similar to those occurring *in utero* where the removal of the oölemma has been described as "a swelling and dissolving process, which gives to the external surface a mammillated aspect."[2] As the dissolving process in the acid medium continues, the zona pellucida changes from a well formed, elastic capsule to a sticky, flaccid, rubbery covering. The changes in the zona as it dissolves in an acid medium resemble those seen in the dissolution of solid gelatin in warm water. As the zona dissolves, it grows thinner, becomes film-like and disappears. The dissolving process can be observed as a series of gradual changes in the zona in the less acid solutions (pH 4 to 5), in which it takes from one to 24 hours depending upon the concentration of HCl. In the more acid fluids (below pH 3.7) the dissolution of the egg membrane takes place within a few minutes. In a few cases the zona disappeared from eggs placed in Ringer's fluid at pH 5.4, but the results obtained with fluids above pH 4.3 were not uniform.

The above results indicate that sufficient acidity in the uterine environment would effect the necessary changes in the zona pellucida to permit the growing blastocyst to expand, and eventually would bring about the complete removal of the zona. It is significant in this respect that hydrogen ion concentrations as low as pH 5.7 have been measured in deciduomata of the rat and that such decidual tissue surrounds the implanting blastocyst.[3]

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[2] Defrise, A., *Anat. Rec.*, **57**, 239.

[3] Hall, B. V. In press.

7844 P

Subnormal Temperatures from Poisoning in Relation to Toxicity Determination.

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It seems important, especially in connection with toxicity determination, to point out that toxic doses of many substances may produce fall of temperature before death or recovery. An old clinical direction calls for note of the body temperature in case of poisoning and special arrangements if needed to reduce heat loss. Incidentally, the statement, "quinine has no significant effect on the normal temperature in man or in animals",[1] appears to need revision, the statement, "in toxic doses the fall is proportional to the collapse",[2] being truer. Quinine has produced subnormal temperatures in man,[3] and in the rabbit fall of 12°C.,[4] and the related cinchonine fall of 14°C. in this animal.[5]

We were led to note rectal temperatures in guinea pigs, used for preliminary toxicity determinations on cinchona bases, by unwittingly repeating an old observation,[6] finding live animals, sometimes quite active, cadaverously cold to the touch. The drugs were given subcutaneously in the flank, in doses mostly in the lethal range, which is for most of these bases 100-400 mg. per kg. Using an average of about 7 animals in each series, we found in the case of each of 22 cinchona bases at least one animal in each series showing fall of 6° C. or more before death or recovery. The phenomenon seems common to all members of the group, including quinine, and is not limited to the cinchona series, for we found it also with morphine and quinoline, and Louvier[7] has noted it with phenobarbital.

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†This work has been aided by a grant from the Committee on Scientific Research of the American Medical Association.

1 Pharmacology and Therapeutics, A. R. Cushny, 10th Ed., 1934, C. W. Edmunds and J. A. Gunn.

2 Manual of Pharmacology, T. Sollman, 4th Ed., 1932.

3 Cordell, E. F., *Maryland M. J.*, 1880, **6**, 257. Roberts, A. E., *Lancet*, 1895, **1**, 644. Rosenberg, D. D., *Med. Rec.*, 1902, **62**, 151. Lavier, G., *Bull. soc. path. exot.*, 1931, **24**, 184.

4 Hogyes, A., *Arch. exp. Path. u. Pharm.*, 1881, **14**, 113.

5 Dupuis, L., *Thèse de Paris*, 1877. Dupuis, L., and Laborde, J. V., *Tribune Med.*, 1878, **10**, 147.

6 Grimaux, E., and Laborde, J. V., *Compt. rend. soc. de biol.*, 1892, **44**, 608.

7 Louvier, M., *Arch. di farmacol. sper.*, 1932, **54**, 68.

The fall is not necessarily premortal: thus, one of our animals recovered completely after fall of 9°C. from hydrocinchonine; Simpson[8] reported recovery of a monkey after fall of 24.6°C. from ether anesthesia and chilling. Shivering is occasionally seen. Fall is not always immediately associated with collapse: a quinidine-injected animal with temperature 31.3°C. moved around at this time quite normally and drank water.

Partly as a result of these observations, one of us (W.T.D.) has since been led to accumulate data on determination of comparative toxicity, *e. g.*, the toxicity of a substance such as quinidine, capable of producing serious fall in temperature, is measured against that of a "standard" related substance, such as quinine, injected in doses toxicologically comparable, on the same day or days and as nearly as practicable just before or after, so that the heat loss conditions are substantially the same for the animals of both groups over the succeeding period during which death or recovery occurs.

7845 C

Nutritional Value of Human Milk, Cows' Milk and Goats' Milk.

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Prolonged feeding with pure milk leads to a series of anemic conditions which generally are classified under the term of nutritional anemia of infancy (MacKay[1]). Different reactions of the organism to various kinds of milk have been observed clinically and experimentally, and attempts have been made to separate the types of goat milk anemia and cow's milk anemia from the simple anemias of breast-fed infants (Baar[2]). But the formerly believed identity between the anemic conditions caused by goats' milk and cows' milk has been disputed lately by various authors (Brouwer[3]), and the experimental work of Rominger and coworkers[4] seems to justify a doubt in the identity of both forms of nutritional anemias. In our

[8] Simpson, S., *J. Physiol.*, 1902, **28**; *Proc. Phys. Soc.*, xxxvii.

[1] MacKay, H. M. M., Nutritional Anæmia in Infancy, London, 1931, p. 9.

[2] Baar, H., *Beih. Jahrb. f. Kinderheilk.*, 1927, **16**, 1.

[3] Brouwer, E., *Jahrb. f. Kinderheilk.*, 1923, **102**, 257.

[4] Rominger, E., Meyer, H., and Bomskov, C., *Z. f. d.*, 1933, **89**, 786.

present series of experiments we studied under the best possible observance of equal conditions the effect of feeding human milk, cows' milk and goats' milk upon the development and the blood picture of growing albino rats.

Each of 8 large and 2 small litters of our normal breeding stock including 78 animals was divided into 3 groups and the animals fed with equal amounts of human milk, cows' milk and goats' milk. The human milk was obtained from the Obstetrical Wards of the State Charity Hospital and collected daily with a glass breast pump into a pyrex flask. The goats' milk was furnished twice a day by 2 goats which were kept on the hospital grounds and fed with dry food and fresh grass. The goats were milked directly into a chemically clean pyrex container. The young rats were put on the milk diet after weaning at an age of 4 weeks and at an approximate weight of 20-25 gm. Each rat was kept in a separate cage of galvanized iron and the body weight, erythrocyte count and hemoglobin content of the blood were determined at weekly intervals. Stained blood smears were examined repeatedly for pathological cell forms and *Bartonella muris*. As soon as an animal succumbed, an autopsy was performed and the organs fixed in formalin for histological study. Adult animals fed with the specific type of milk and young rats, which were killed by means of starvation and vitamin deficiency, served as controls for the pathological findings on the milk-fed young animals.

Our results are compiled in Table I.

TABLE I.

Diet	No. Litters	No. Animals	No. Anemic Litters	No. Anemic Animals	No. Dead Animals
			%	%	%
Human milk	6	17	0 (0)	0 (0)	0 (0)
Cows' "	8	28	3 (37.5)	7 (25)	2 (7.1)
Goats' "	8	33	7 (87.5)	26 (78.7)	18 (54.5)

None of the animals fed with human milk became anemic and only 37.5% of the litters of our breeding stock showed anemia after prolonged feeding with cows' milk. Only 2 out of 28 animals died from cows' milk anemia during an observation period of 20 weeks. In contrast to those findings, goats' milk produced in 7 out of 8 litters a severe anemia and over half of the animals (54.5%) died in their course. In the same litters of animals and under the same experimental conditions goats' milk produced therefore an anemia in a higher percentage and of a more severe degree than the cows' milk.

Table II gives the compiled figures of body weight, hemoglobin content of the blood and erythrocyte count of each of the 3 groups on milk diets.

TABLE II.

Diet	Determinations of	Normal Values	Weeks of Milk Diet 2	3	4	5	6	7	8	9	10
Human Milk	Weight (gm.)	24	32	36	43	61	66	74	78	96	89
	Hemoglobin (gm.)	9.7	9.7	11.1	10.6	10.1	10.9	10.0	9.5	9.5	9.2
	Erythrocytes (millions)	5.31	5.9	7.1	7.4	7.7	7.9	8.3	8.3	8.4	8.6
Cows' milk	Weight (gm.)	26	44	46	51	62	83	68	83	84	90
	Hemoglobin (gm.)	9.3	7.1	6.2	5.7	4.8	3.9	too low to read			
	Erythrocytes (millions)	5.1	4.4	4.4	3.6	3.1	2.9	2.4	2.0	1.9	2.0
Goats' milk	Weight (gm.)	24	35	51	51	49	48	46	65	69	—
	Hemoglobin (gm.)	9.2	6.8	5.8	4.5	3.8	3.2	too low to read			
	Erythrocytes	5.5	3.2	3.1	2.7	2.3	2.8	2.6	2.0	2.3	—

The rate of growth as represented by the curve of the body weight determinations seems to be more rapid in the animals fed with cows' milk and goats' milk than with human milk. Later, however, as the effects of the anemia become apparent, the body weight of the animals fed with goats' milk and cows' milk comes to a standstill or even a decline in the weight curve can be noted. The effect of the anemia upon the gastrointestinal tract of the animals must be taken as the cause of this phenomenon.

The hemoglobin content of the blood remains on an equal level in the group fed with human milk and declines rapidly in the groups fed with cows' milk and goats' milk. No principal difference between both groups can be noted. After 5 to 6 weeks of diet the hemoglobin level is too low to give accurate results with the Newcomer method of determination.

The number of erythrocytes rises slowly but continuously in animals fed with human milk. The decrease of erythrocytes is more rapid in the group fed with goats' milk and in the group fed with cows' milk although after a certain time no difference between the erythrocyte counts in both groups can be noted. This fact explains the slight hyperchrome character of the goats' milk anemia during the first weeks as compared with the distinct hypochrome character of cows' milk.

Summary and Conclusions. The feeding of various types of milk to young albino rats of the same litter has a different effect

upon the growth, the erythrocyte count and the hemoglobin content of the animals. Goats' milk produces in many instances a severe anemia of hyperchromic character, cows' milk produces a distinctly hypochromic anemia in a smaller percentage of the animals, and human milk does not have any deleterious effect upon the growth or the red blood picture of the growing albino rat.

7846 C

Further Evidence of Insusceptibility of the Rat to a Dietary Deficiency of Vitamin C.

CAROLINE TUM SUDEN AND OTIS E. ALLEY. (Introduced by F. H. Pratt.)

From the Department of Physiology, Boston University School of Medicine, and the Evans Memorial, Massachusetts Memorial Hospitals.

Svirbely[1] has reported that the adrenal gland and liver tissues of rats and mice, reared for several generations on a diet devoid of vitamin C, were rich in the anti-scorbutic substance. As far as one can ascertain from the literature, the vitamin C requirement of the rat had been investigated chiefly from the standpoint of growth and fertility, since the characteristic scorbutic lesions such as osteoporosis, sub-periosteal hemorrhages, and petechiae of cutaneous and mucous surfaces had not been observed in this species.[2] Moreover, such observations had not been extended, as a rule, to more than one generation. Therefore in order to obtain further evidence that the rat requires no dietary supplement of vitamin C, the incisors of animals maintained and reared for 3 generations on scorbutic diet were examined histologically according to the method of Höjer,[3] as one of the most specific and sensitive criteria of the scorbutic process.

A small series of 6 female and 7 male rats was placed on a diet of boiled whole milk and dried bread. This diet, tested on guinea pigs, was found to be scorbutic; but in other respects complete and adequate. The substitution of fresh whole milk not only prevented and cured scurvy, but maintained guinea pigs in good health. After

[1] Svirbely, J. L., *Biochem. J.*, 1933, **27**, 960.

[2] Harden, A., and Zilva, S. S., *Biochem. J.*, 1918, **12**, 408; Drummond, J. C., *Biochem. J.*, 1919, **13**, 77; Parsons, H. T., *Biochem. J.*, 1920, **14**, 587; Kennedy, *Quart. J. Exp. Physiol.*, 1926, **16**, 281; Hartwell, G. A., *Biochem. J.*, 1930, **24**, 967; Güdjonsson, S. V., *Biochem. J.*, 1930, **24**, 1591.

[3] Höjer, A., *Brit. J. Path.*, 1926, **7**, 356.

6 months of this regime the group was bred, and the offspring in turn raised and bred on the C-deficient diet. Of the 3 generations so obtained, the lower incisors were examined histologically for evidence of scurvy. The average ages of the f_0, f_1, f_2 groups were respectively 12 to 15, 5½, and 2 months. In no instance did the sections show any deviations from the normal tooth structure. The organization of the odontoblasts, the process of dentine formation and the vascularity and cellular content of the pulp cavity were in no way disturbed. Furthermore, from gross examination the adrenals showed no signs of hypertrophy nor any diminution in the speed of reduction of silver nitrate (0.4% solution). Consequently there was no evidence reflecting a latent or early disturbance resultant from the continuous omission of vitamin C from the diet.

7847 P

Blood Grouping of the Rwala Arabs.

WILLIAM M. SHANKLIN. (Introduced by G. H. Miller.)

From the Department of Histology, School of Medicine, American University of Beirut, Syria.

Although many investigators have made blood typings on Asiatics in both the Near East and the Far East, no studies have been previously reported on the inhabitants of the Syrian desert. The subjects of this study all belong to the Rwala tribe whose customs and manners have been fully elucidated by Musil.[1] Being entirely nomadic and very powerful, the Rwala penetrate the deepest parts of

TABLE I.
Percentage of distribution of blood groups among Rwala Arabs.

Camp	No. Persons	% in each group				Frequencies			Racial Index
		O	A	B	AB	p	q	r	
1*	79	94.94	5.06	0	0	.21	.00	9.74	0
2	77	87.01	9.09	2.60	1.30	.54	.17	9.33	2.66
3	58	82.76	12.07	5.17	0	.63	.27	9.09	2.33
4	59	72.88†	13.56	11.86	1.69	.79	.68	8.53	1.12
5	47	63.83†	21.28	12.77	2.13	1.24	.76	7.99	1.57

* Since the Rwala are nomadic a camp number is designated instead of naming the locality.

† Decrease in O due to negro slave admixture.

[1] Musil, A., The Customs and Manners of the Rwala Bedouins. American Geographical Society, Oriental Explorations and Studies, No. 6, 1928.

the desert. Except for some admixture with their negro slaves, the tribe is relatively pure as they retreat into the inner desert in cases of outside invasion. The blood samples were collected and typed on the same day against sera from donors belonging to group A and B. All agglutinations were checked by microscopical observation.

The high percentage of group O among the Rwala is wholly unexpected and most striking. The decrease in O in some of the camps is in direct proportion to the amount of admixture with the negro slaves in those particular camps. Although those frankly negro were excluded from the series, half-breeds were included. No negroes were found in camps 1 and 2, whereas in camp 5, the headquarters of the emir, large numbers of slaves are present.

In surveys of the incidence of blood group frequencies among peoples of the Near East by Parr,[2, 3] the highest percentages for group O reported for Syrian Arabs is 37.82%; Palestinian Jews, 37.18%; Armenians, 28.14%; Turks, 36.8%; and Egyptians, 24.0%. The handbook of Steffan[4] also shows that all other previously reported Asiatics have a low incidence of group O.

Snyder,[5] on the basis of blood groups, divides mankind into 7 types, one of which is the Pacific-American. This type, in contradistinction to all the other types, is characterized by a high percentage of group O, being above 90% in many pure tribes of American Indians. Very obviously the Rwala are to be classed with the Pacific-American type, thereby raising the question concerning the significance of this relationship. Snyder[5] says: "It has been assumed by most writers that the human race was originally all of group O, the factors A and B having arisen separately by later mutation." As the American Indians are very high in group O, it is generally conceded that they migrated from some part of Asia before the mutation of factors A and B. The writer suggests a similar explanation may also apply to the Rwala Arabs. The variation in the percentages of group O in the various camps in no way invalidates the results of this study, as the variations are obviously due to mixture with negro blood.

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[2] Parr, L. W., *Am. J. Phys. Anthrop.*, 1931, **6**, 15.

[3] Kappers, C. U. A., and Parr, L. W. An Introduction to the Anthropology of the Near East. 1934, Amsterdam.

[4] Steffan, P. *Handbuch der Blutgruppenkunde.* 1932, Munich.

[5] Snyder, L. H. Blood grouping in relation to clinical and legal medicine. 1929, Baltimore.

7848 C

Effect of Cortico-Adrenal Extract on Leucocytes in Blood of Normal Adult Rabbits.*

C. A. FOX AND R. W. WHITEHEAD. (Introduced by Ivan E. Wallin.)

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Rogoff and Stewart,[1] Baumann and Kurland,[2] Britton and Silvette,[3] Swingle, Pfiffner, Vars, Botts, and Parkins,[4] and Harrop, Weinstein, Soffer, and Trescher,[5] have shown that adrenalectomy in animals produces a marked increase in blood concentration. So great is the dehydration that a false erythemia is seen, the red blood cell count in the blood showing an increase of from 50 to 100%.[6] Extensive changes are also encountered in the leucocytes in the blood as shown by Zwemer and Lyons.[7] Adrenalectomy in their animals resulted in a decrease in the total number of leucocytes in the blood of which the polymorphonuclear neutrophiles were markedly diminished, while the lymphocytes were slightly increased in number. Corey and Britton,[6] studying this problem more intensively, reported a concomitant decrease of about 50% in the white blood cell count in cats following adrenalectomy, with a blood picture resembling agranulocytic angina. The administration of cortico-adrenal extract to their adrenalectomized animals with a granulocytopenic blood picture, brought about a complete restoration of normal cell values in the blood. Wenner and Cone,[8] using extracts of the adrenal cortex in human pyogenic infections, were unable to observe any effect on the white blood cell counts or Schilling indices; the blood picture in their patients improved with the general improvement in the condition of the patients.

The purpose of this investigation was to ascertain the effect of the administration of cortico-adrenal extract, prepared according

*Aided by a grant from the Committee on Therapeutic Research of the American Medical Association.

[1] Rogoff, J. M., and Stewart, G. N., *Am. J. Phys.*, 1926, **78**, 711.

[2] Baumann, E. J., and Kurland, S., *J. Biol. Chem.*, 1927, **71**, 281.

[3] Britton, S. W., and Silvette, H., *Am. J. Phys.*, 1931, **96**, 15.

[4] Swingle, W. W., Pfiffner, J. J., Vars, H. M., Botts, P. A., and Parkins, W. M., *Science*, 1933, **77**, 58.

[5] Harrop, G. A., Weinstein, A., Soffer, L. J., and Trescher, J. H., *J. Exp. Med.*, 1933, **58**, 1.

[6] Corey, E. L., and Britton, S. W., *Am. J. Phys.*, 1932, **102**, 699.

[7] Zwemer, R. L., and Lyons, C., *Am. J. Phys.*, 1928, **86**, 545.

[8] Wenner, W. F., and Cone, A. J., *Arch. Otolaryng.*, 1934, **20**, 178.

tc the method of Swingle and Pfiffner, upon the white blood cell count and the differential leucocyte count in normal rabbits. The normal white blood cell counts and differential counts in the rabbits were determined by studying the blood during 3 days prior to the injections of the extracts. The blood was taken from the ear vein at the same hour each morning to rule out, as much as possible, the diurnal variation in the leucocytes in the blood stream.

Twenty-four adult rabbits were studied in this experiment. Of these, 10 received 1 cc. of cortico-adrenal extract daily; 6 received 1 cc. of normal isotonic saline daily; 2 received 1 cc. of cortico-adrenal extract inactivated by heat; and 6 were normal untreated controls. The rabbits were injected intramuscularly for a period of 30 days. During the first 4 days after the beginning of the injections, complete white blood cell counts and differential leucocyte counts were done. From the fourth day to the completion of the experiment, the blood was studied at intervals of 4 days.

The experimental results show no change in the leucocytes in the blood after prolonged cortico-adrenal extract administration to normal adult rabbits. The white blood cell counts and the differential leucocyte counts were well within normal limits during the time of this experiment.

7849 P

Reaction of Ovaries of Mature Female Rats to Injections of Oestrin.*

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Twenty-five mature female rats received daily injections of 200 rat units of a concentrated oestrus-inducing extract† for 8 to 15 days (a majority were for 12 days). Vaginal smears usually revealed a complete cornification of the vaginae for the first 3 to 5 days of the experiment which did not persist throughout the injection period except in 2 rats. At autopsy it was found that the

* These studies were aided by a grant from the Division of Medical Sciences of the Rockefeller Foundation.

† Progynon-B, described by the manufacturers, Schering Corporation, as a benzoic acid ester of dihydro-follicular hormone. A portion of this material was furnished gratuitously by Schering Corporation, Bloomfield, N. J.

ovaries of these rats presented a normal number of corpora lutea but that the corpora were greatly increased in size when compared with those of rats killed during the normal oestral cycle. They were, however, similar in appearance and size to the corpora lutea of rats killed during the latter half of pregnancy. The pituitary glands of the oestrin-injected rats were increased in weight. Their mean weight was 19.2 mg.; the range was from 12.5 to 27 mg., and all but 2 weighed above 15 mg. The mean pituitary weight of 143 normal cyclic female rats was 10.5 mg.

Serial sections of all ovaries and representative sections of the uteri and the vaginae were cut. For control material similar sections were made of the ovaries and the accessory organs of 30 female rats killed during the normal oestral cycle and from 25 rats killed during the latter half of pregnancy. The relative size of the corpora lutea in the various groups was obtained by measuring the 2 greatest diameters, in millimeters, of the sections of the corpora lutea with a micrometer eye-piece. The product of the 2 greatest diameters of the corpora was calculated and the average of the 5 greatest products thus obtained was considered as the size of the corpora lutea in the ovary.

Our results indicate that the corpora lutea present in the ovaries of rats receiving oestrin were definitely and consistently larger than those found in the ovaries of normal rats killed during the oestral cycle but that they are equal in size to those found in the ovaries of rats killed during the latter half of pregnancy. In the normal rats the mean product of the 2 diameters of the corpora lutea was 0.9 mm. The range was from 0.5 mm. to 1.4 mm. In 19 of the 30 rats used, this product was below 1 mm. In both the pregnant rats and those receiving oestrin, the mean products of the 2 diameters of the corpora were 2.6 mm. In both groups the extreme range was from 1 to 3.9 mm., but in 80% of the rats of both groups this product was between 2 and 3.9 mm. From these data it seems legitimate to conclude that injections of large amounts of oestrin into mature female rats induced a very marked increase in the size of the corpora lutea. Hisaw and his associates[1] have recently reported that oestrin stimulates the production of a luteinizing factor of the anterior hypophysis. Although we have approached the problem from another angle, our results confirm those of these investigators.

The vaginae of all the pregnant rats were mucified. In 3 exper-

[1] Hisaw, F. L., Fevold, H. L., Foster, M. A., and Hellbaum, A. A., *Anat. Rec.*, 1934, **60**, 52 (Supplement).

imental rats in which the corpora were not greatly increased in size the vaginae were cornified and in 5 rats which presented extremely large corpora lutea the vaginae were definitely mucified. In the rest of the experimental rats the vaginae were stratified, in some of these, there was some evidence of early mucification.

Detailed morphologic studies on the anterior pituitaries of these rats are incomplete. However, in the glands that we have studied the basophiles were reduced in relative percentage and those present were enlarged and devoid of granules. The eosinophiles were moderately reduced in percentage and many cells exhibited evidence of granular loss. The reduction in percentage and morphologic changes in the eosinophiles were most marked in those anterior lobes in which the weight increase was greatest. In previous reports we have invariably associated granular loss from the eosinophiles with the presence of active corpora lutea in the ovaries. The results presented in this paper confirm these previous findings.

7850 P

Production of a Collateral Circulation to the Heart.

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The experiments recorded in this paper are to a large extent an outgrowth of work done previously.[1, 2] It was our purpose to destroy the normal coronary circulation and in its place provide an adequate collateral circulatory bed. This study, begun in February, 1932, is based upon 103 dogs in which attempts were made to produce a collateral circulation to the heart. The problem of producing a new blood supply to the heart resolved itself into 2 components, (1) to provide a vascular bed from which blood vessels could grow directly into the myocardium, (2) to reduce the circulation in the myocardium, so that collateral circulation in the adhesions might develop. The operative procedure was one of almost constant evolution. Omitting the details of development the procedure used was as follows:

[1] Hudson, C. L., Moritz, A. R., and Wearn, J. T., *J. Exp. Med.*, 1932, **56**, 919.
[2] Moritz, A. R., Hudson, C. L., and Orgain, E. S., *J. Exp. Med.*, 1932, **56**, 927.

Epicardiectomy* was done under ether anesthesia by abrasion and stripping with a special burr. The lining of the parietal pericardium was removed. Silver clips were placed around the major coronary arteries. The lumina of these vessels were reduced in stages at repeated operations over a period of 2 years in some of the animals.

The collateral bed established through the pericardial adhesions was injected with a watery suspension of iron ferrocyanide (0.4%). The injection was carried out as follows: The venae cavae and azygos vein were ligated. The right auricle was incised. The aorta was clamped close to the heart and the aorta was incised between heart and clamp. This dissection prevented injection of the heart by way of the aortic coronary ostia and by way of the thebesian vessels. Dye was run into the right carotid artery under gravity pressure. The pressure in the arterial system, as determined from the left carotid artery, was between 100 and 120 mm. mercury. The injection was continued for one-half hour. It was concluded that the dye that entered the heart in such injection experiments arrived by way of the extracardiac anastomoses with the coronary system.

Normal control dogs have extracardiac anastomoses with the coronary system as does the human being. In normal dogs, injected as described, a little dye can be found in the coronary arteries, in the auricular wall, in the myocardium and in the fat at the base of the heart. The amount of dye in the myocardium is minimal and except for the major coronary arteries is sometimes altogether absent.

When a collateral vascular bed is supplied to the dog's heart, as was done in these experiments, the coronary arteries can be completely occluded without producing infarction or fibrosis of the myocardium. The dog recovers after total coronary occlusion is produced, is normally active and seems to be in good health. We obtained recovery in dogs with total occlusion of the right coronary artery, with total occlusion of the descending branch of the left coronary artery or with total occlusion of the circumflex branch of the left coronary artery. In one dog recovery occurred after complete occlusion of the right coronary artery, complete occlusion of the ramus descendens at the bifurcation of the left coronary artery and almost complete occlusion of the circumflex branch of the left coronary artery. The reduction in this experiment was estimated to be at least 85% of the total cross sectional area of the 2

* In so far as we know this is the first time this word has been used in medical literature and the first time this operative procedure has been done.

coronary arteries. The dog appeared to be in good health. The heart after injection through the extracardiac anastomoses contained as much dye as did the somatic muscles. In this animal a collateral blood supply had been built up sufficient to maintain cardiac function.

The experiments show that a collateral blood supply to the heart of dogs can be made available by operation. The circulatory bed thus supplied does not interfere with the filling and emptying of the heart. This vascular bed distributes blood supply to the myocardium and experimentally becomes a mechanism which permits compensation that protects a faltering heart from stopping. It makes possible the maintenance of function despite the occlusion of large coronary trunks.

7851 P

Influence of Diet on Lipid Content of the Rat's Brain.

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Previous studies on the blood lipids in epilepsy[1] and in acute infectious diseases[2] indicated to us the need for further information regarding the factors which control the lipid content of the tissues. The present report deals with the influence of various diets on the total fatty acid, cholesterol and phospholipid content of the rat's brain. The interest of earlier workers appears to have centered chiefly in cholesterol. Page and Menschick,[3] Chanutin and Ludewig[4] and others have shown that the cholesterol content of the rabbit's liver is greatly increased by prolonged ingestion of this substance, whereas that of the brain is not changed. Best and Ridout[5] found that the livers of cholesterol-fed rats could be prevented from becoming excessively fatty by addition of choline to the diet. A high fat diet is said to inhibit and a high carbohydrate diet to accelerate deposition of cholesterol in the liver.[4] The effect of diet on

[1] McQuarrie, Irvine, Husted, Clara, and Bloor, W. R., *J. Clin. Invest.*, 1933, **12**, 255.

[2] McQuarrie, Irvine, and Stoesser, A. V., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1281.

[3] Page, I. H., and Menschick, W., *J. Biol. Chem.*, 1932, **97**, 359.

[4] Chanutin, A., and Ludewig, S., *J. Biol. Chem.*, 1933, **102**, 57.

[5] Best, C. H., and Ridout, J. H., *Am. J. Physiol.*, 1933, **105**, 6.

the phospholipid content of the brain appears not to have been investigated.

A series of experiments covering a wide range of diets and extending over periods of from 1½ to 13½ months proved fairly definitely that the lipid content of the growing rat's brain tends to remain strikingly uniform. Between 3 and 6 normal rats from our own stock colonies were placed at the age of 3 weeks on one of the following special diets: 1. High-carbohydrate (80%), 2. high-fat (85%), 3. high-protein (80%), 4. low-fat (20%), 5. fat-free (Burr and Burr), 6. standard stock diet (Jackson), 7. standard control diet (Chanutin and Ludewig), 8. the latter plus 2.5% added cholesterol, 9. the same plus 5% added cholesterol, and finally, 10. the standard control diet plus 1.25% cholesterol and 1.30% choline. For each group of animals on special diet a control group was kept on a standard mixed diet for the same period of time. At the end of the experimental period the animals were killed by sudden decapitation, after which the brain was immediately dissected out *in toto*. One-half of the organ was then carefully weighed before and after complete desiccation in a drying oven for determination of the water content. After preparation by the method of Osato and Hehi[6] with minor modifications, the remaining half was used for determination of the total fatty acids, cholesterol and phospholipids by the methods of Bloor.[7, 8]

In spite of the extreme variations in diet there was no significant difference between the composition of the brains of the experimental animals and that of the controls, with the exception of the 44-day experiment on the effect of added cholesterol. In the latter instance the brains of rats receiving the standard diet plus 2.5 or 5.0% added cholesterol showed average cholesterol values which were higher and phospholipid values which were lower than those for the control group, although the water content did not differ. The average cholesterol percentage (wet weight) for the control group was 1.32 and the phospholipid 6.98 as against cholesterol 1.65 and phospholipid 5.58 in the experimental animals. This difference entirely disappeared, however, when the experiments were continued beyond 3 months. The total lipids were found to be unaffected by diet. Age was found to be a definite factor in the cholesterol content of the brain. In keeping with the results of previous workers, the percentage of cholesterol was found to increase significantly with age.

[6] Osato, S., and Hehi, M., *J. Biol. Chem.*, 1930, **87**, 543.

[7] Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

[8] Bloor, W. R., *J. Biol. Chem.*, 1929, **82**, 273.

7852 C

Changes in Temperatures of Tissues After Systemic Applications of Short Wave Electric Fields.

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The apparatus used in these investigations is a product of the Research Laboratories of the General Electric Company and, through the courtesy of this company, was made available to us for experimental work. Essentially, the apparatus is a short wavelength radiogenerator, the output of which is fed to large condenser plates rather than to an antenna. The electric energy is available over a wavelength range of 7.5 to 20 meters, with an approximate power output of 150 to 400 watts. Throughout these investigations we have used a frequency of 27.5 million cycles per second (wavelength of 10.9 meters).

Measurements of temperatures of tissues have been made by means of special detachable thermocouples incorporated in the electromotive thermometer.[1] Rectal temperatures were obtained by means of an automatically recording resistance thermometer (Brown Instrument Company).

The application of energy between the primary condenser plates of the oscillator to dogs with distemper—the plates of the oscillator extended along the body of the animals from neck to knee in all these experiments—showed that the rectal temperatures increased from 1.5° to 2°C. under a divided dosage of approximately 10 minutes of radiation over a 20-minute interval. The increase of temperature was uniform per unit of applied electric energy until a rectal temperature of 40°C. (104°F.) was obtained; after that the increase of temperature per unit of energy decreased. In dogs with distemper, it was found that mucus was mobilized markedly during the first few treatments in the electric field. After five or six exposures of the animal to radiothermy, no mucus was in evidence.

One animal was given the usual 10-minute body treatment in the electric field. The rectal temperature rose 1°C., and within 5 minutes after discontinuation of the treatment the temperature began to drop exponentially with the passage of time. The following day this same animal was anesthetized by means of an intravenous injection of sodium amytal (sodium isoamylethylbarbiturate). Given an identical 10-minute treatment, the rectal temperature of the dog

[1] Sheard, Charles, *Am. J. Clin. Path.*, 1931, **1**, 209.

rose 2.5°C. and remained at this peak for 30 minutes before the exponential loss of heat began to occur. Measurements on temperatures of tissues of the upper part of the leg of this animal showed that the intramuscular and subcutaneous temperatures paralleled the rectal temperature after treatment, but that the intramuscular temperature remained approximately 0.5°C. above the rectal, whereas the subcutaneous temperature remained about 0.2°C. below the rectal. Similar results were obtained in other dogs.

Measurements of temperatures of tissues were made on a series of dogs in which a general hyperthermia was produced by means of the short wave field. Before treatment, the intramuscular temperature ordinarily was found to be slightly higher than the rectal temperature, whereas the subcutaneous temperature was usually slightly lower than the rectal. The intramuscular temperature of the upper part of the legs of the dogs was generally found to be between 39° and 39.5°C. (102.2° and 103.1°F.) and approximately 1°C. higher than the subcutaneous temperature. With thermocouple needles in the tissues during oscillation of the field, both intramuscular and subcutaneous temperatures of the tissues rose rapidly. When application of the field was discontinued, both tissue temperatures dropped steeply along an approximately exponential path. However, the intramuscular temperatures remained constantly above the subcutaneous values, and while the subcutaneous temperatures dropped to the rectal level, or slightly lower, within 5 to 10 minutes after discontinuation of the treatment in the electric field, the intramuscular temperatures remained constantly above the rectal temperatures.

In a number of experiments the changes of temperature induced in the tissues at the knee joints of dogs were compared with those induced in the muscle tissues of the upper part of the leg. The normal intra-articular temperature was approximately 2°C. less than the intramuscular temperature. When thermocouples were present in the tissues during oscillation of the high-frequency field, the rise of temperature in the intra-articular regions was much sharper than in the intramuscular tissue, but the subsequent fall in temperature was also more rapid. Five minutes after the treatment had been discontinued, the intra-articular temperature attained a fairly constant value which remained approximately 1°C. below that attained by the intramuscular temperature. There was a decided increase in the intra-articular temperatures as compared to both rectal and intramuscular temperatures.

Small holes were drilled in the bone of the leg of a dead animal.

Measurements of temperature were made before and after exposures of the limb to the short wave field. The temperature of intramuscular tissue was found to be 32°C. (89.6°F.); temperatures of bone in 2 different regions were found to be 30°C. and 33°C. (86° and 91.4°F.) respectively. Applications of the high frequency field for 5 minutes produced an increase of 1.2°C. in the temperatures

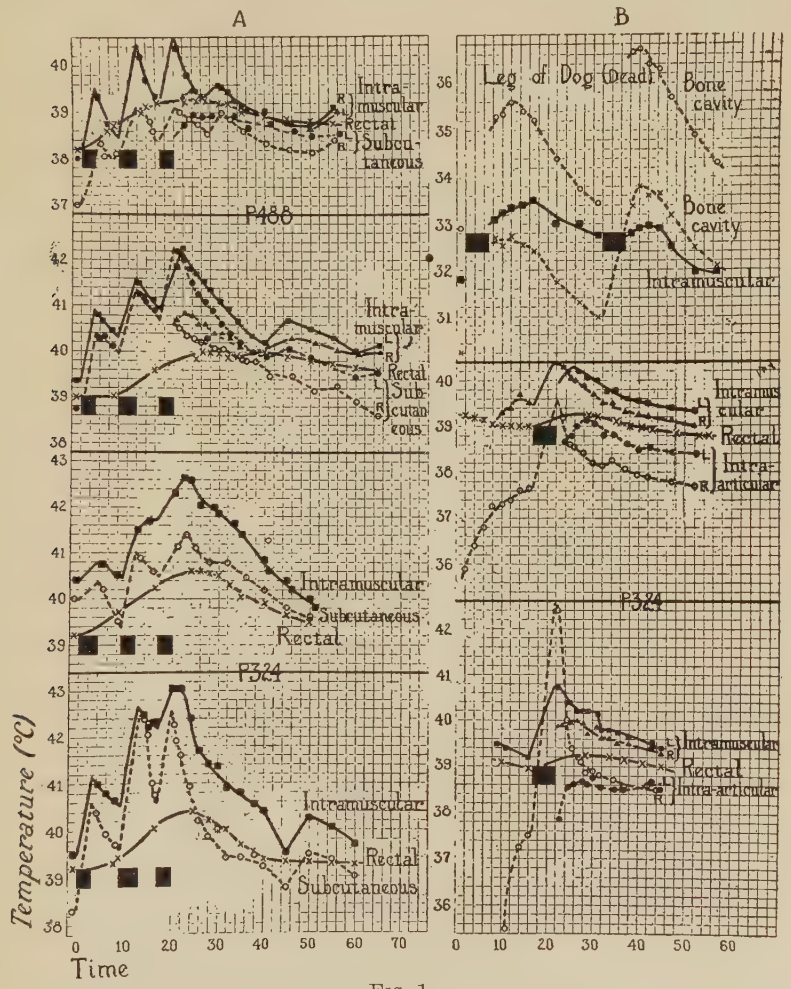

FIG. 1.

Curves showing the comparative heating of living tissues in the high-frequency field. Part *A*. Effects on intramuscular and subcutaneous temperatures. Part *B*. Effects on intra-articular (knee joint) and intramuscular temperatures as compared with rectal temperatures. Each group of curves shows the changes in temperature obtained when thermocouples were inserted in the tissue (1) throughout the period of application of the field and (2) after the cessation of application of the field. Time of treatment and chronological order are indicated by the blackened rectangles.

of muscle tissues and of 3.3°C. in the temperatures of both portions of the bone under test.

Conclusions. These experimental data show that, in living animals, the temperatures of certain different types of tissues are affected to different degrees by the systemic application of the short-wave electric field. Muscle tissue is affected slightly more than is subcutaneous tissue; intra-articular tissue is affected more than is muscle tissue. Furthermore, it has been shown that the reactions of anesthetized animals to general hyperthermia produced by this physical agent (short electric wave fields) are quantitatively different from those produced in unanesthetized animals. Measurements of temperatures of tissues of dead animals have shown that bone is heated much more markedly than is muscle tissue and they have indicated that the changes of temperature produced by this high frequency field are strictly dependent on the position and constitution of the various tissues.

7853 C

Thermal Changes Produced in Tissues by Local Applications of Radiothermy.

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The short electric wave generator developed in the Research Laboratories of the General Electric Company was used in these investigations. In utilizing the energy of this high frequency field for the investigations of thermal changes produced by local applications of the field, small insulated condenser plates were attached by means of tubular telescopic rods to the large condenser plates.

Measurements of temperatures were made (series of 30 dogs) on the intra-articular and subcutaneous tissues of both knee joints of each animal after local application of the short wave field to the region of the knee joints. Condenser plates 2.5 by 3 inches, separated by distances which varied from 2.5 to 4 inches, produced the high frequency field into which was placed the part of the animal to be tested. The current density applied to the knee joints varied from 0.1 to 0.05 ampere per square inch of surface of the small auxiliary condenser plates. The frequency of oscillation of the electric field

(which is not markedly affected by considerable variations in the distance and arrangements of the auxiliary plates) was 27.5 million cycles (wave length of 10.9 meters).

In order to evaluate the errors in the measurement of temperature produced by the introduction of a thermocouple needle into tissue and the errors caused by the presence of such a needle in tissue during application of the short wave field, the following procedure was carried out Thermocouple needles were introduced into the intra-articular and subcutaneous (at knee joint) tissues of one leg, and measurements of the temperatures were obtained over a 10-minute period. The leads were then detached from the needles, and the needles were left in position in the tissue while the short wave energy was passed through the knee joint. After cessation of the treatment, leads were again attached to the thermocouples and the measurements of the temperatures were taken over a 30-minute period. Immediately following this period, the short wave field was applied for the same length of time to the knee joint of the second leg of the animal. In this case the thermocouples were inserted immediately after the high frequency treatment had been completed, and the measurements of temperature were taken again over an interval of 30 minutes.

It was thus possible to ascertain the temperatures produced by application of the short wave field to tissue in which thermocouple needles were present during treatment. These temperatures theoretically should be somewhat higher than those of normal tissue in which no metallic needle is present. By delaying the insertion of the thermocouple needles into the second leg of the animal until after application of the high frequency field, it was possible to obtain readings of the temperatures produced by application of the short wave field to normal tissues, with, however, the recognition of the physical fact that these temperature readings were subject to losses due to the introduction of a low temperature needle of some thermal capacity and of considerable thermal conductivity. The temperatures thus obtained should be somewhat lower than the true temperatures of the tissues. By obtaining, on each animal, measurements of temperature by both of these methods (one somewhat higher than the true temperature and the other slightly lower than the true temperature) it is possible to estimate the effect of the high frequency field on the temperatures of tissues to a high degree of probable accuracy.

In 2 control dogs, examined in this manner and without subjection to the short wave field, significant changes in the intra-articu-

lar temperatures were not observed, but the subcutaneous temperatures rose slowly during the period that the thermocouple needle was inserted in the tissue. The average rectal temperature for the 30 dogs was 39.1°C. (102.3°F.). The average intra-articular temperature before application of the short wave field was 36.8°C. (98.2°F.). The average subcutaneous temperature in the region of the knee joints was 36.1°C. (96.9°F.). The rectal temperatures were not affected by local treatments of short duration.

Eleven dogs were subjected to the localized short wave field across the knee joint for 2 minutes, with a plate distance of 4 inches and a current density of 0.05 ampere per square inch. When thermocouples were present during treatment the intra-articular temperature rose 3.5°C. to a point slightly above the rectal temperature, whereas the subcutaneous temperature rose 2.5°C. but remained below the rectal temperature. These temperatures dropped sharply after cessation of the treatment. When thermocouples were inserted after application of the electric field, the intra-articular temperature rose about 1°C. and dropped very slowly with time, whereas the subcutaneous temperature apparently was not at all affected by the treatment. Two dogs were treated in this same field with an exposure of only one minute. The results for measurements with thermocouples present during oscillation of the field were practically the same as those stated in the foregoing, but there was no change in temperature indicated by thermocouples inserted after treatment in both the intra-articular and subcutaneous measurements.

Two dogs were treated for one minute in a field with a plate distance of 2.5 inches and a current density of 0.09 ampere per square inch. The effects on the thermal changes of tissue as indicated by thermocouples, inserted during and after the oscillation of the field respectively, were identical. The intra-articular temperature rose 4.5°C. to a value well above the rectal temperature, and decreased approximately exponentially with time after treatment. The subcutaneous temperature rose 5.5°C. to a value above both rectal and intra-articular temperatures.

The remaining 13 experimental dogs were treated under a constant distance of 3 inches between the plates of the auxiliary condenser and under a current density of 0.08 ampere per square inch.

Six dogs were treated for one minute through each knee joint. When thermocouples were present during application of the electric field, the intra-articular temperature rose 4°C. and the subcutaneous 3°C. Both temperatures were well above the rectal value and

both fell very rapidly after cessation of treatment. When thermocouples were inserted after application of the field, the rise of

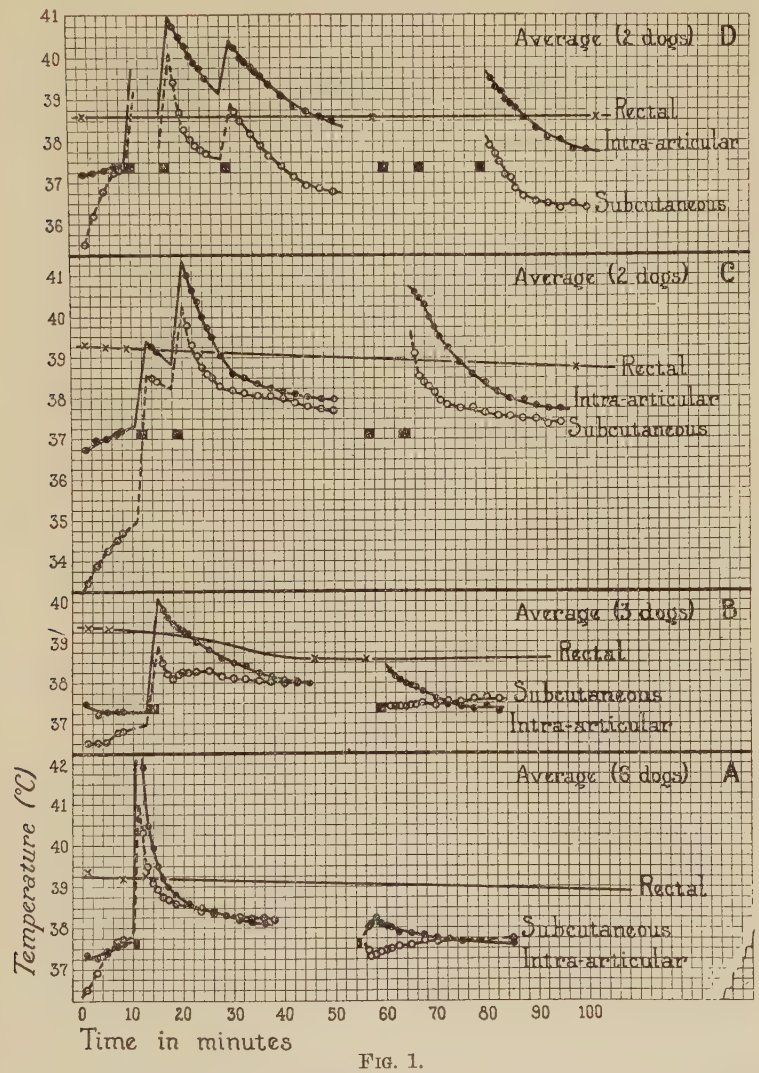

FIG. 1.

Curves showing the effects produced by the application of localized high frequency fields to the region of the knee-joints of dogs. (Current density of 0.056 to 0.11 ampere per square inch, frequency of 27.3×10^6 cycles per second, treatment plates insulated and separated 3 inches.) The curves give the intra-articular, subcutaneous and rectal temperatures after the application of varying doses. In obtaining the curves of the left portion of the diagrams, thermocouple needles were inserted in one leg of the animal throughout the treatment; in the right portion, the thermocouples were inserted in the other leg of the same animal after the cessation of the field. Time of treatment and chronological order are indicated by the blackened rectangles.

intra-articular temperature was only 0.4°C. and the subcutaneous temperature was apparently not affected.

Three dogs were treated for 2-minute intervals in this same fashion. By the first method of measurement, the increase in intra-articular temperature was 2.5°C. and the increase in a subcutaneous temperature 1.5°C.; both temperatures fell slowly with the passage of time after treatment. By the second method of measurement, the intra-articular temperature rise was 1°C., but the subcutaneous temperature again appeared to remain constant.

Two dogs were treated in this same manner by 2 applications of the field, each for 2 minutes, with an interval of 5 minutes between applications. In these experiments the results of measurements with thermocouples present during oscillation of the field and of measurements with thermocouples inserted after application of the field were identical. The intra-articular temperature rose 4°C. and returned in an approximately exponential fashion to a constant value at the end of the 30 minutes of observation. The subcutaneous temperature rose 3°C. and paralleled the drop of the intra-articular temperature. Both temperature values exceeded the rectal temperature at their peak. Two dogs were treated in the same way, but an additional 2-minute exposure to the electric field was made 10 minutes after the second exposure. Again the temperatures obtained by both methods of measurement were practically identical and qualitatively similar to those described.

Summary. It is apparent that errors in the measurement of temperature, due to the presence of a metallic needle (thermocouple, without leads) in tissue during exposure of that tissue to a high frequency electric field are greater when the time of exposure is short and when the intensity of the electric field is low. The relationship of the heat produced in deep-lying tissues to the heat produced in superficial tissues is dependent on the distance at which the condenser plates are placed with respect to the locations of the tissues. This fact is of importance in the therapeutic uses of high frequency fields (radiotherms). When a considerable air space separates these plates from the surface of the tissue, the change in temperature produced in the deep-lying intra-articular tissue is greater than that produced in the subcutaneous tissue. The converse relationship maintains when the plates are placed close to the surface of the tissue. This dielectric layer effect is superimposed on any specific heating due to difference in constitution of tissue and variation in wave length of radiation which may be present. Schlie-

phake[1, 2] first demonstrated this type of depth effect of the high frequency field, and has presented experimental evidence which indicates that high frequency treatment plates which are allowed to make contact with the surface of tissue produce a tissue heating which is closely comparable with that of diathermy, in which the production of heat is predominantly in the superficial layers of tissue.

The experimental evidence presented clearly demonstrates that it is possible to produce abnormally high temperature in a chosen region (*i. e.,* knee joint of a dog) by means of local applications of short wave electric energy of sufficient intensity. Furthermore, these relatively high temperatures may be produced in the deep (intra-articular) tissue of the region without the simultaneous production of high temperatures in the superficial (subcutaneous) tissues of the region (*i. e.,* portion of leg of dog) exposed to the high frequency electric field of the type used in these investigations.

7854 P

Precipitation of Apparent Creatinine from Serum Ultrafiltrates.

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In earlier isolation experiments[1, 2] on blood creatinine, the use of protein precipitants, of adsorbents, and evaporation in acid solution all made the interpretation of results difficult. In the case of sera showing various degrees of nitrogen retention a simpler path which avoids these difficulties is open. In the following experiments cellophane No. 300 was used as the membrane in an ultrafilter operated by a nitrogen pressure of 400 lb. per square inch. A few drops of toluene were added to the serum and the receiving vessel.

To 10 cc. of ultrafiltrate are added 250 mg. of pure picric acid. This is dissolved by shaking the tube under the hot water tap, and the solution is cooled to about 25° in cold water. One-tenth cc. of 10% potassium chloride solution is added, and the solution is mixed at once. An excess of picric acid may precipitate at this point, but unless

[1] Schliephake, Erwin, *Klin. Wchnschr.*, 1928, **7**, 1600.
[2] Schliephake, Erwin, *Strahlentherapie*, 1930, **38**, 655.
[1] Gaebler, O. H., and Keltch, A. K., *J. Biol. Chem.*, 1928, **76**, 337.
[2] Gaebler, O. H., *J. Biol. Chem.*, 1930, **89**, 451.

the amount of apparent creatinine is very large, one can centrifugalize and decant without any loss of chromogenic substance. A small crop of crystals then forms on the bottom or wall of the tube containing the decanted liquid. These crystals contain the apparent creatinine, but free picric acid and a small amount of potassium picrate are still present. If the concentration of apparent creatinine is very high, crystallization may go to completion in a few hours, but as a rule 24 hours are required, and with amounts below 4 mg. per 100 cc. the time required may be still longer.

TABLE I.

Source of Serum	Apparent creatinine in mg. per 100 cc. of ultrafiltrate	
	Before Precipitation	After Precipitation
Human, nephritis	13.6	2.7
" "	9.0	2.3
" renal calculi	2.8	1.6*
Dog 10, uranium nephritis	13.3	2.8
Dog 11, " "	4.2	1.8
Same animal, 2 days later	4.1	2.0
" " recovering	2.7	2.0*
Creatinine added:		
Normal serum + 3 mg./100 cc.	3.9	1.5*
" " + 3 mg./100 cc.	4.6	1.5*
" " + 11.8 mg./100 cc.	12.2	0.9

* 5 to 6 days required for complete precipitation.

The apparent creatinine values in the ultrafiltrate before and after carrying out the above precipitation are shown in Table I. Unless otherwise stated, 24 hours were allowed for precipitation. Values below 5 mg. per 100 cc. were determined at 1:5 dilution, others at higher dilutions, the concentration of picric acid being kept at 1.2%, and the technique being the same as in Folin's original method for determination of creatinine in picric acid blood filtrates. Analyses of the precipitate in three or four instances confirmed the results obtained before and after precipitation, but most of the precipitates are being used for the more important matter of qualitative examination. The cause of the slow precipitation in the case of ultrafiltrates from sera to which small amounts of creatinine had been added is not clear. The filtrates which were used gave no turbidity with picric or tungstic acid, but on shaking a slight froth appeared which broke almost instantly. Use of a heavier or denser membrane[3] may be indicated.

It must not be assumed from the writer's earlier studies dealing with fractions of ordinary filtrates, that all of the chromogenic

[3] McBain, J. W., and Kistler, S. S., *J. Gen. Physiol.*, 1928, **12**, 187.

substance precipitated in the present experiments is creatinine potassium picrate. A direct study of the precipitate will be reported later.

7855 P

Amplified Heart Sounds. Use of the Crystal Microphone.*

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The object of this paper is to describe a practical method of amplifying heart sounds that may be used to teach students abnormalities of these sounds, to distinguish murmurs and to elucidate cardiac arrhythmias. This procedure is particularly useful in teaching auscultation to students.

Many amplifiers† have been described both in the United States and abroad. These usually have been bulky and not portable and many require the use of batteries. We have designed a high-gain 3-stage amplifier that is portable, inexpensive, operates on alternating current and does not require the use of batteries. The essential features consist of a type 57 tube of high amplification value, which is resistance coupled to another 57 tube in the second stage; and this in turn is resistance coupled in a 2A5 tube in the third stage. The power supply is the customary circuit and is heavily filtered using 30 henry chokes and 28 microfarads of condenser. A 2-ampere fuse was installed to prevent burning out the transformer in the event the amplifier is plugged into direct current by mistake. A 12-inch permanent magnet speaker was selected because of a better tone response than the magnetic speaker, and because it is lighter in weight than the dynamic speaker. An output transformer, while not required, was found to improve the tone response. It is possible with certain modifications to use this amplifier with direct current.

* The authors wish to acknowledge their indebtedness to Mr. G. Ryan, Chicago representative of the Turner Company of Cedar Rapids, Iowa, for the microphone used in their work.

† The crystal type of microphone has been used by Dr. H. B. Weiss at the Cincinnati General Hospital and a crystal microphone was used in the apparatus developed and demonstrated by Mr. M. L. Lockhart at the meetings of the American Heart Association and the American Medical Association. The advantage of the arrangement described in this report is its inexpensiveness.

The choice of a suitable microphone presented the greatest problem in our work. The aim of a portable and inexpensive outfit ruled out the condenser microphone as it is bulky and requires a separate power supply. The same is true of the ribbon and dynamic types of microphones. The carbon microphone is noisy and the inability to use it in any position, due to shifting of the carbon granules, proved it to be unsatisfactory. Theoretical and practical work demonstrated that the piezo crystal microphone was well adapted for our work. It is light, portable, inexpensive, does not require the use of any special transformer or batteries, and will operate in any position without any background noise or hiss. It is sensitive to the frequency level of heart sounds (50 to 160 vibrations per sec.).

Clinical tests with this amplifier have shown it capable of picking up the normal heart sounds and the usual murmurs in most individuals, unless these sounds are distant, and reproduces them through the loud speaker in sufficient volume to be audible in a room of moderate size, without any distracting hum. Further work is in progress with increased stages of amplification which are necessary to reproduce faint heart sounds.

7856 P

Acoustic Function in Pouch Young of the Opossum.

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Pouch young of the opossum were tested for reflex responses to acoustic stimuli, using a litter captured with the mother when they had attained an estimated age of 29 days (40 mm. C. R. length). The tests were begun when the litter was estimated 43 days old (63-64 mm. C. R.). Preliminary tests for vestibular reflexes show that these appear in pouch young of about 41 days (61-62 mm. C. R.), in general agreement with Langworthy.[1] The experiments on acoustic reflexes were begun on 5 young in the brood pouch and attached to the nipples in the normal manner. The pouch was opened only enough to permit observation of the young, the mother being held quietly on her back. Other factors than the acoustic stimuli were eliminated. During the course of the experiment 2 of the young were killed for neuro-histological study of the labyrinth and

[1] Langworthy, Orthello, *J. Comp. Neur.*, 1928, **46**, 201.

brain, so the later results were obtained on 3 experimental animals. As controls we used the mother of the pouch young and another litter of 4 free young 146 mm. long (C. R.) at the beginning of the experiments.

Preliminary tests consisted of shrill whistling, sharp handclaps, vibration of a tuning fork at C″ and rustling of the straw of the pen. These were applied as close to the pouch young as possible without introducing other factors such as rush of air, etc. A wind instrument known as the "organette", giving the notes C, E. G, C′, E′, G′, C″, E″, G″ and C‴, was used in a closer analysis of sounds which elicited reflex responses. The chords C′ E′ G′ C″ and E G C′ on this instrument were also used. Sustained notes could be produced when desired.

The mother responded to all tests save the tuning fork by twitching of the abdominal and other muscles. The control litter of free young responded to all tests save the tuning fork by startled movements. It was concluded that the volume of sound produced by the tuning fork was insufficient to bring forth responses. The same note, namely C″, from the "organette" elicited responses in the mother, the control litter and in pouch young beginning at 51 days of age. Rustling in the straw brought forth the most marked response of all stimuli from the mother and the control litter, but no response from the pouch young. It is a composite sound of small volume and the responses observed are tentatively regarded as conditioned reflexes.

No response to any of the acoustic stimuli was observed in the pouch young until they were 50 days old (71 mm. C. R.). The auditory canal was patent from about the 43rd day after birth. At 50 days the young responded by a definite contraction of the trunk musculature, simulating Coghill's total reaction pattern,[2] to shrill, sharp whistling. At 51 days the response to whistling was somewhat doubtful but there was a strong trunk movement when the shrill E′ note was sounded. Slight responses were obtained at this stage also to G″ and C″, but not to other notes. At 52 days E″, C′, G and the chord C′ E′ G′ C″ brought forth slight to moderate reflex movements, while C″, G′ and E′ elicited strong reactions of the trunk. At 53 days G″, E and C‴ also elicited trunk movements. At 55 days C and chord E G C′ brought forth similar responses.

In addition to the trunk reflexes the 55-day stage showed a movement of the pinna when middle C was sounded. At 56 days E′ and

[2] Coghill, G. E., Anatomy and the problem of behaviour, Cambridge University Press, 1929.

E″ elicited movement of the pinna, in addition to trunk movements, while C, E, G, C′, G′, C″ and G″ elicited similar movements of the tail, in addition to the trunk reflex. The pinna and tail responses are regarded as individuated reflexes, in Coghill's sense. At 57 days the tail movements were not observed, but most of the notes were followed by trunk and pinna reflexes.

It will be observed that notes of high pitch elicit the earliest reflexes and that, in general, the appearance of responses to other notes, as development progresses, is in the order of the descending musical scale to C. No tests have been made as yet below this note.

Histological study of the cochlea of pouch young at several stages of development shows that the organ of Corti differentiates gradually from base to apex. Terminations of cochlear fibers are present about the bases of the hair-cells as early as 29-day pouch young. The organ of Corti as a whole is, however, in an early stage of differentiation, and, as above stated, is non-functional at this stage. By the stage of 50-day pouch young it shows marked histological differentiation and the beginning of functional activity. This is long before the young normally leave the brood pouch, and, presumably, before they have any occasion to respond to acoustic stimuli.

The earlier responses to high notes are correlated with the differentiation of the organ of Corti in the more basal part of the cochlea, while the later responses to low notes are attained only when histological differentiation has reached the apex of the organ of Corti. The fiber tract to the midbrain acoustic center is well shown in pyridine-silver series of the brain of a 43-day specimen. It is present long before reflex responses to acoustic stimuli appear.

Peripheral end-organs and central pathways of the acoustic system, similar in general pattern to those of the opossum have been described in the frog tadpole,[3] but no experimental evidence with respect to their capacity to function has yet been obtained.

[3] Larsell, O., *J. Comp. Neur.*, 1934, **60**, 473.

7857 C

Effect of Methylhistamine and Hydroxyethylglyoxaline on Gastric Secretion and Blood Pressure in the Dog.

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Prior to the work reported in this paper histamine (0.5-1.0 mg.), pilocarpine (5-10 mg.), and iso-pilocarpine (10 mg.) were the only imidazoles out of 10 examined that stimulated gastric secretion.[1, 2]

Through the courtesy of Dr. Pyman of the Boots Pure Drug Company of Nottingham, England, samples of 2 other imidazoles, namely, methylhistamine (4.5 methylaminoethylglyoxaline dihydrochloride) and 4,5 hydroxyethylglyoxaline, were supplied to us for physiological investigation.

With a recently devised method Pyman has succeeded in the synthesis of methylhistamine which has properties altogether different from those reported by him and Fargher[3] for this supposed formerly synthesized compound. The oxytocic activity of methylhistamine on the isolated guinea pig uterus, instead of being about 1% of that of histamine, as recorded in their original paper, was now found by them to be almost as great as that of histamine itself.

We have studied the effect of these 2 imidazoles upon gastric secretion and the blood pressure in the dog. It was found that subcutaneous injections of doses of histamine and methylhistamine, which contained equivalent quantities of base, caused a definitely parallel response in the stimulation of gastric secretion and acidity in Pavlov pouch dogs. (Table I.) Methylhistamine likewise caused a drop in the blood pressure of the dog quantitatively

TABLE I.

Effect of Histamine, Methylhistamine and Hydroxyethylglyoxaline on Gastric Secretion in Pavlov Pouch Dog.*

Dog	Histamine		Methylhistamine		Hydroxyethylglyoxaline	
	Vol. of G Sec. (cc) Mean	Total HCl Output (mg.)	Vol. of G Sec. (cc) Mean	Total HCl Output (mg.)	Vol. of G Sec. (cc) Mean	Total HCl Output (mg.)
1	28.2	130.24	29.0	114.05	0	0
2	12.4	62.75	11.7	40.50	0	0

* Only secretion above basal recorded in this table.

1 Sacks, Ivy, Burgess and Vandolah, *Am. J. Physiol.*, 1932, **101**, 334.

2 Burgess and Ivy, PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 115.

3 Pyman and Fargher, *J. C. S.*, 1921, 731.

similar to that caused by histamine. Hydroxyethylglyoxaline showed no stimulation of gastric secretion nor did it affect the blood pressure in the dog. This is apparently due to the absence of either the methyl or ethyl-amino group from this compound.

Conclusions. (1) Methylhistamine and histamine are equally potent in stimulating the gastric glands to secrete acid. (2) Methylhistamine and histamine cause a similar fall in the blood pressure in the dog. (3) Hydroxyethylglyoxaline has no effect upon gastric secretion and blood pressure in the dog.

7858 P

Body Temperature Changes Produced by Sodium Salts of Some Aromatic Acids.*

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Concerning the relations between the chemical constitution of the nitrophenols and their specific action on cellular metabolism Heymans[1] makes the following statements:

"In the dinitrophenol the stimulating action on metabolism is associated with position 1 to 4 of one of the NO_2 radicles, the greater the distance between the two NO_2 radicles the greater is the hyperthermic action. The introduction of one or more aliphatic CH chains up to the pentyl chain, progressively increases the specific action of the dinitro-derivatives as catalyzers of cellular respiration. The stimulating action on cellular metabolism is decreased by the addition of an NH_2 radicle to the benzyl chain and is abolished by the addition of an HSO_3 radicle."

3-5 dinitrosalicylic acid which has the two NO_2 groups in the desired positions relative to each other and hydroxyl group offers the opportunity to study the effect on body temperature when a COOH group is substituted into a dinitrophenol.

Sodium 3,5 dinitrosalicylate injected intramuscularly into pigeons and subcutaneously, intramuscularly or intraperitoneally into rats, in the range dosage 25 to 133 mg. per kilo body weight, does not produce a rise in body temperature, but on the contrary

* This research was aided by a grant from the Hendricks Research Fund.

[1] Heymans, C., *J. Pharm. and Exp. Therap.*, 1934, **51**, 144.

produces a distinct and sustained decrease. The maximum decrease (2° to 4°C.) is reached about 2 hours after injection and the return to normal is slow—4 to 7 hours.

The symptoms produced by the larger doses were shivering, retching and vomiting in the pigeons and shivering and drowsiness in the rats.

Assuming that the nitro groups were in a measure responsible for the greater decreases produced by sodium dinitrosalicylate than are produced by sodium salicylate we compared the temperature decreasing effects of sodium 3,5 dinitrobenzoate and sodium benzoate.

Sodium 3,5 dinitrobenzoate causes definite decreases in body temperature of normal pigeons and rats but is not as effective as sodium dinitrosalicylate and the similar symptoms produced are less severe.

In 12 control experiments using untreated animals and animals injected with solutions of antipyrine or sodium salicylate or sodium benzoate, temperature variations less than 2°C. were obtained in all cases.

In 11 pigeon and 10 rat experiments using sodium dinitrosalicylate and in 6 pigeon and 11 rat experiments using sodium dinitrobenzoate, marked depressant effects (more than 2°C.) on body temperature were produced.

7859 C

Low Basal Metabolic Rates Obtained by Low Calorie Diets in Coronary Artery Disease.

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From the Cardiovascular Service and the Medical Services of the Mount Sinai Hospital, New York.

That a diminished food intake results in lowered basal metabolic rates has been known for many years. It is only necessary to mention the classical work performed by Benedict[1] and reviewed by Lusk.[2] In view of the fact that the basal metabolic rate is raised in congestive heart disease, tachycardia, severe dyspnea, and in dias-

1 Benedict, F. G., Niles, W. R., Roth, P., and Smith, H. M., Carnegie Institute of Washington, 1919, Publication 280.

2 Lusk, G., *Physiol. Rev.*, 1921, **1**, 523.

tolic hypertension,[3] we wished to determine the rates of patients who had sustained an acute coronary artery occlusion and who were placed on a diminished food intake.

An adequately balanced 800 calorie diet containing 80 gm. of

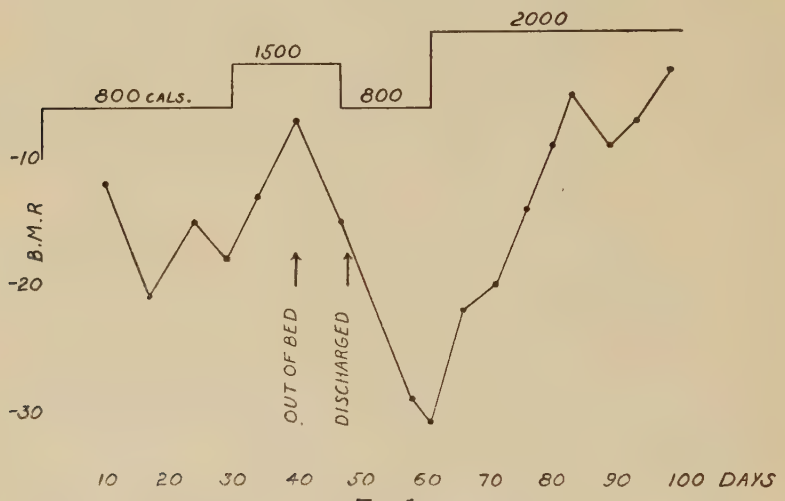

FIG. 1.

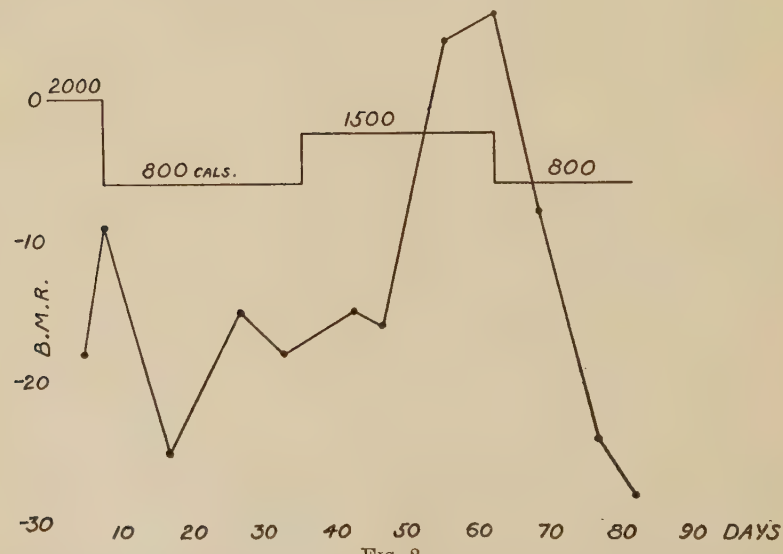

FIG. 2.

[3] Peabody, F. W., Meyer, A. L., and DuBois, E. F., *Arch. Int. Med.*, 1916, **17**, 980. Hamburger, W. W., and Lev, M. W., *Am. Heart J.*, 1925, **1**, 240. DuBois, E. F., Basal Metabolism in Health and Disease, Lea and Febiger, 1924, 306. Boas, E. P., and Shapiro, S., *J. Am. Med. Assn.*, 1925, **84**, 1558.

carbohydrate, 50 gm. of protein, and 30 gm. of fat was prescribed. Care was taken to include adequate vitamins. The fluid intake was limited to 1,000-1,200 cc. per day. Basal metabolic rates were determined frequently and control readings were obtained either at the beginning of the experiment or when the patient's diet was increased. In no case was there any pulmonary congestion or evidence of myocardial decompensation at the time readings were made. In addition to the 7 patients, (Cases I to VII) who suffered from coronary thrombosis we are including one case of severe anginal syndrome caused by coronary artery sclerosis (Case VIII). The latter was always ambulatory and , although a sick man, travelled to and from our clinic. After discharge from the hospital the patients returned regularly for the basal metabolism test.

Graphs of the first 4 patients followed 3 or more months are presented as well as tables of all 8 cases. In the latter for the sake of brevity only the basal metabolic readings characteristic of each

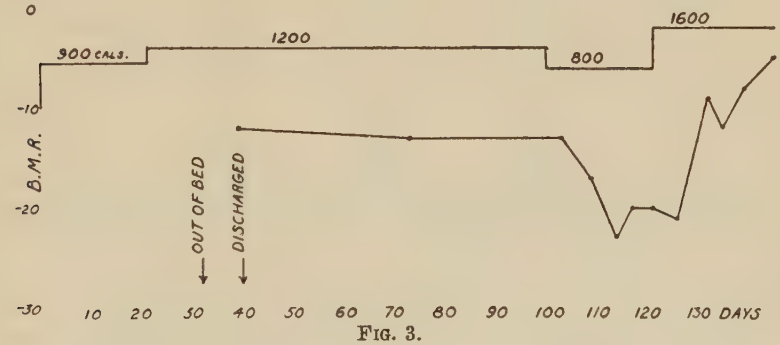

FIG. 3.

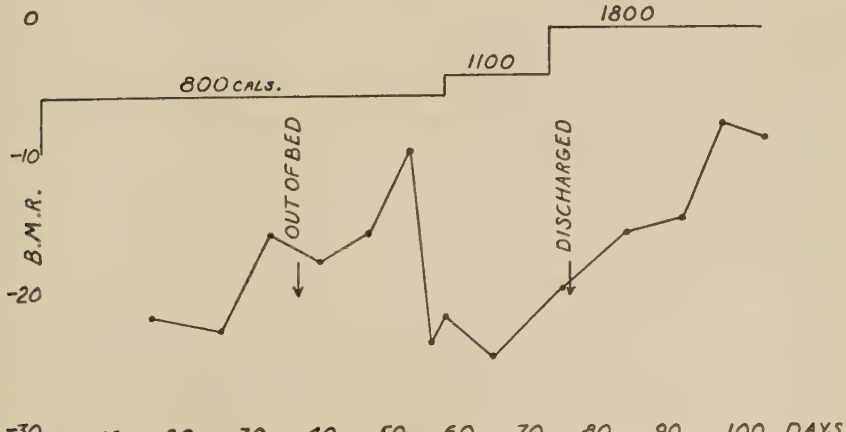

FIG. 4.

TABLE I.

	Sex	Age	Diet	Days on Diet*	Wt.	BMR
1.	M.	59	800	17-29	132	—15 to —21
			1500	10-17	130	— 7 to —15
			800	9-12	131	—29 to —31
			2000	19-37	139	— 3 to — 9
2.	M.	50	Reg.	8	165	— 9
			800	9-25	158	—15 to —25
			1500	20-27	160	+ 4 to + 6
			800	14-25	155	—19 to —28
3.	M.	47	1200	20-99	108	—13
			800	14-20	104	—20 to —23
			1600	18-23	107	— 5 to — 8
4.	F.	44	800	16-64	136	—16 to —25
			1800	33-39	139	— 8
5.	M.	52	800	11-19	153	— 4 to — 6
			800	25-33	146	—10 to —19
			2000	20-35	152	+ 2 to + 8
6.	F.	57	800	5-25	114	— 8 to —15
			800	30-44	106	—17 to —29
7.	M.	62	800	16	140	—11
			800	24-57	132	—18 to —22
8.	M.	62	Reg.		230	— 3
			800	27	213	— 3
			800	30-45	203	—21 to —25

* Indicates the number of days on the diet specified, during which time the designated range of basal metabolic rates occurred.

particular level of feeding are recorded. It will be observed that in 2 to 4 weeks, the basal metabolic rate ranged between —20 and —30%.* In the first 3 patients we lowered and increased the food intake on several occasions with a corresponding decrease or rise in the basal metabolic rate. On return to a regular diet the basal metabolic rate rose to normal, usually in about 1½ to 2½ weeks. Rest in bed, walking about the ward, and even slight to moderate activity after discharge from the hospital did not affect the result. (None of our patients has as yet recovered sufficiently from his disease to be able to return to work.) In other words, the decrease in basal metabolic rate was caused by the diminished caloric intake.

Much interest has been aroused by the operation of complete thyroidectomy advocated by Blumgart, Levine and others[4] for the

* The rates are based on the DuBois normal standards as modified by Boothby and Sandiford, *Am. J. Phys.*, 1929, **90**, 291.

[4] Blumgart, H. L., Levine, S. A., and Berlin, D. D., *Arch. Int. Med.*, 1933, **51**, 866.

treatment of patients suffering from congestive heart failure or the anginal syndrome. These authors have obtained low basal metabolic rates in their patients. In fact, their object seems to be to keep the basal metabolic rate of their patients between —20 to —30%, results that we obtain on an 800 calorie diet. It is therefore suggested that this diet may produce over a period of one to 3 months or more, results comparable to those obtained by complete thyroidectomy over a long period of time.

It appears that in a patient with coronary artery thrombosis kept on an 800 calorie diet the basal metabolic rate may be lowered just as in a normal person.

A lowered basal metabolic rate is associated with a diminished velocity of the blood flow and a decreased amount of work of the heart. Hence low caloric diets should help patients with myocardial impairment. Lusk[2] and DuBois[5] have already discussed this theoretical phase. One of us (A.M.M.) has been treating his coronary thrombosis patients by bed rest and an 800 calorie diet for 7 years with excellent results.

7860 C

Peroxidases and Cell Activity in Developing Egg (Orthoptera).*

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From the Zoological Laboratory, State University of Iowa.

Cellular activity during normal embryonic development of the common grasshopper, *Melanoplus differentialis,* at constant temperature (25°C.) is characterized by 3 distinct periods: (a) a period of rapid cell proliferation (pre-diapause), (b) a period of developmental block or cellular inactivity in which mitosis, growth, etc., are absent (diapause), (c) a period of marked differentiation and growth terminating in the hatching of the embryo (post-diapause).[1] It becomes of some interest, therefore, to determine physiological changes which accompany the various phases of cellular activity. The present discussion has to do with results of studies on the peroxidase reaction during the entire course of embryonic development.

[5] DuBois, E. F., *Bull. N. Y. Academy of Medicine,* 1933, **8**, 680.

* Aided by grant from Rockefeller Foundation for work on cellular physiology

[1] Bodine, J. H., *Physiol. Zool.,* 1932, **5**, 549.

TABLE I.
Guaiac Reaction of Embryos in Different Stages of Development.

Pre-diapause			Diapause			Post-diapause		
Developmental age at 25°C. days	Guaiac Reaction		Developmental age at 25°C. days	Guaiac Reaction		Developmental age at 25°C. days	Guaiac Reaction	
	Eggs at constant temp. 25°C.	Eggs exposed to 5°C*		Eggs at constant temp. 25°C.	Eggs exposed to 5°C*		Eggs at constant temp. 25°C.	Eggs exposed to 5°C*
1	—		21	+—	+	50	++	++
4	—		22	+—	+	51	++	++
6	—	+	**23**	+—	+	52	+++	+++
8	—	+	24	+	+	53	+++	+++
10	—	+	25	+	+	54	+++	+++
11	—	+	**26**	++	++	55	+++	+++
12	—	+	27	++	++	56	+++	+++
13	—	+	**28**	++	++	60	+++	+++
14	—	+	30	++	++	hatching	+++	+++
15	—	+	32	++	++	nymph	—	—
16	—	+	35	++	++			
17	—	+	38	++	++			
18	—	+	40	++	++			
19	+—	+						
20	+—	+						

— = negative reaction.
+— = faint reaction.
+ = positive reaction.
++ = strongly positive reaction.
+++ = very strongly positive reaction.
* After developing at 25°C. for days shown.

In each test the eggs (usually 10 in number) were crushed with glass rods in narrow test tubes. Small amounts of distilled water (1 cc.) were next added. After the addition of a few drops of hydrogen peroxide, tincture of guaiac was added, the contents of the tubes shaken and the tubes allowed to stand at room temperature until the characteristic blue color appeared. Check experiments were run in which pH values were controlled by phosphate buffers (pH 7.3) but since the eggs are themselves buffered no significant differences were noted between samples buffered with and without phosphates. Several hundreds of eggs were used and each was carefully examined so that both chronological and morphological histories could be accurately determined. This procedure, as will be noted below, is of much importance in attempting a correlation between cellular and physiological behavior of the embryos.

A summary of results is given in Table I. It will be noted that for pre-diapause eggs kept at constant temperature (25°C.) no positive peroxidase reactions are obtained until the 19th or 20th day when diapause or cellular block normally occurs.[2] During diapause the reaction gradually increases, reaching a maximum as diapause disappears. In the post-diapause period, with rapid differentiation and growth, the reaction reaches a maximum intensity just before hatching. Curiously, after the embryo hatches no positive reactions are obtained.[3] If pre-diapause eggs are subjected to low temperatures (0-5°C.) for appropriate periods of time (1 month) and then tested, a positive reaction is always obtained. Obviously, exposure to low temperature in some way or other destroys an inhibitory mechanism which at normal temperature (25°C.) prevents the reaction.

Thus it appears that the peroxidases are, at constant temperature (25°C.), normally present in the embryonic cell from the time of fertilization but are inhibited (as judged by negative guaiac reaction) by some mechanism already present in the cell. The potency of this inhibitor at constant temperature (25°C.) gradually diminishes until at diapause or developmental block the peroxidase reaction normally becomes positive. Exposure to low temperature (0-5°C.) during this period (pre-diapause) in some way or other destroys the inhibitor and a positive peroxidase reaction is then obtained.

Additional evidence supporting such a concept is furnished by the results of mixing extracts of pre-diapause eggs kept only at constant

[2] Slifer, E. H., *J. Morph.*, 1932, **53**, 1.

[3] Bodine, J. H., *Biol. Bull.*, 1925, **48**, 79.

TABLE II.

Inhibition of the peroxidase activity of post-diapause developing embryos by substances contained in pre-diapause developing embryos.

No. of eggs crushed		
Pre-diapause (16 days at 25° C.)	Post-diapause (17 days from hatching)	Guaiac reaction
15	0	—
0	5	+++
15	5	±
20	5	—

TABLE III.

Inhibition of the peroxidase activity of embryos exposed to 5° C. (14 day morphological stage) by substances contained in embryos of similar morphological stages but kept only at 25° C.

No. of eggs crushed		Guaiac reaction
14 day embryos at 25° C.	14 day embryos exposed to 5° C.	
10	0	—
0	10	++
40	10	±

(This inhibition is not a dilution effect. Peroxidases may be demonstrated in extracts of cold treated embryos or of post-diapause embryos diluted 4 to 6 times.)

temperature (25°C.) with those of post-diapause eggs showing marked peroxidase reactions (Table II). Extracts of pre-diapause eggs exposed to 5°C., when added to extracts of similar embryos kept only at constant temperature (25°C.) give the same general result (Table III).

Other characteristics of these enzymes such as cyanide sensitivity, thermolability, etc., point to the fact that they undoubtedly are peroxidases. Positive reactions also indicate the presence of indophenol oxidases and tryosinases in addition to peroxidases. Enzymes are always present in the embryo and not in the yolk.

It has previously been shown[1] that exposure of pre-diapause eggs to low temperatures will also prevent or destroy the factor producing cellular block or diapause. Curiously cellular activity is absent or at a minimum during diapause even though positive peroxidase reactions are found. During pre-diapause development at constant temperature (25°C.) marked proliferation of cells occurs even though negative peroxidase reactions are given. It seems reasonable, therefore, to infer that peroxidases are *not primarily* concerned with cellular activity as shown in this particular type of embryonic cell.

Summary. 1. A study has been made of the peroxidase activity (guaiac test) during the embryonic development of the grasshopper, *Melanoplus differentialis*. 2. At constant temperature (25°C.)

negative peroxidase reactions are found for pre-diapause eggs. Positive reactions are given for diapause and post-diapause eggs, Embryos after hatching give negative reactions. 3. Exposure of pre-diapause eggs to low temperatures (0-5°C.) apparently destroys a naturally occurring inhibitor so that eggs thus treated always give positive peroxidase reactions. 4. Ideas are expressed as to possible manner in which peroxidase inhibitors function. 5. No correlation between cellular and peroxidase activity seems apparent.

7861 P

Action Potentials of the "Respiratory Center."

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A systematic study of localized potentials lends itself well to the localization of the respiratory center and to an analysis of its still unknown mode of function. After removing the skull cap and the cerebral and cerebellar hemispheres in the dog, we explored the depths of the brain stem from the thalamus through the upper portion of the cervical cord with needle electrodes. A variety of potentials were encountered but in the medulla and upper cervical cord discrete potentials, of orderly sequence, associated with the respiratory act, have been definitely established. These potentials are readily counted and appear to arise from individual nerve cells or neuraxones.

Inspiratory potentials commonly accelerate and deaccelerate with the waxing and waning of inspiration. These potentials cease during the phase of expiration, or continue at a lowered rate during this period. As a rule, the amplitude of discrete potentials remains moderately constant, but instances of gross change of intensity associated with little change in rate have been encountered.

Expiratory potentials during active expiration may accelerate and deaccelerate with waxing and waning of the expiratory act. Usually discrete potentials progress at a uniform rate throughout expiration regardless of its duration indicating a tonic nature of discharge. They are inhibited in rate or number, only during the phase of inspiration. These results fit in with classification of types of breathing previously recorded by the potential method in respiratory muscles of the dog (Gesell).

Central expiratory potentials, of the tonic type, were commonly of a much higher frequency than those previously recorded in respiratory muscles (Gesell), suggesting the existence of a step-down mechanism. There is also some evidence that certain potential frequencies may be a multiple of a lower rate of discharge.

Continuously occurring discrete potentials of a uniform frequency have been encountered. These potentials have been changed by inspiratory and expiratory mechanical asphyxia to either the inspiratory or expiratory type.

Electrodes inserted into silent regions may from time to time register respiratory potentials as resting cells come into activity. Frequently these silent regions yielded respiratory potentials during mechanical asphyxias. Potentials emanating from a previously silent region, if from a true respiratory center, indicate that the completeness of participation of the center varies with the magnitude of breathing.

The exact cellular or fiber source of central potentials is being sought by placement of minute lesions at the site of the lead-off electrodes immediately following photographic registration of potentials. The fact that both expiratory and inspiratory potentials can be recorded from one placement of the electrodes, or that one type may give way to the other with a minute displacement of the electrodes, indicates a close anatomical association between the so-called inspiratory and expiratory centers.

7862 C

A New Method of Determining Plasma Fibrin.

SAMUEL ROSENFELD AND ALEXANDER S. WIENER. (Introduced by B. Kramer.)

From the Division of Hematology of the Department of Pathology, Jewish Hospital, Brooklyn, N. Y.

The usual method of estimating the fibrinogen content of the blood is by recalcifying the oxalated plasma in order to convert the fibrinogen into fibrin.[1] The quantity of fibrin precipitated is then determined by the Kjeldahl, the gravimetric, the colorimetric, or the refractometric methods.

[1] Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Williams and Wilkins, Baltimore, 1932. This method of precipitating the fibrin was developed by Cullen and Van Slyke, *J. Biol. Chem.*, 1920, **41**, 587.

The method of determining plasma fibrin presented here is based on the coagulant activity of certain types of snake venom. Martin[2] was the first to demonstrate the striking activity of the venoms of certain Australian snakes in causing coagulation of blood both *in vivo* and *in vitro*. Lamb[3] found that minute doses of such venoms could also coagulate *in vitro* blood which had lost the capacity for spontaneous coagulation on account of the addition of citrates, oxalates or fluorides. Among the venoms noted for their coagulating action are those of the following snakes: *Notechis scutatis* (tiger snake), *Pseudechis porphyriacus* (black snake), *Echis carinata* (phoorsa) and *Viperi russelli* (daboia). The former 2 species are found in Australia and the latter 2 in India. In addition, certain species of the Lachesis group of vipers are known to exhibit this property. In the present investigation, tiger snake venom, which apparently has the most powerful coagulating action,* was used. In all probability, the venoms of the other species mentioned can also be used.

The actual technique of estimating the concentration of fibrinogen (as fibrin) in the plasma with the aid of tiger snake venom is as follows: One cc. of oxalated plasma is diluted 25 to 30 times with normal saline solution. (The quantity of powdered potassium oxalate used for collecting the blood need not be accurately measured.) To the diluted plasma is added 0.3 cc. of a 1 to 5,000 dilution of dried tiger snake venom in normal saline. Coagulation usually occurs within 2 or 3 minutes. The clotted plasma is transferred to dry filter paper on a funnel. When practically all the fluid has drained through, distilled water is poured on the filter, whereupon the fibrin clot will usually float free in one piece. The distilled water is allowed to remain in contact with the clot for 10 minutes, after which time the water is drained off. This process of washing the clot is repeated 4 or 5 times, when the clot should be colorless. The entire clot can then be picked up by twisting it on a glass rod. The clot is transferred to a piece of dry filter paper, and all the fluid remaining is gently expressed. It can then be thoroughly dried on a watch glass kept in an incubator at 37°C. over night, or in a hot air oven at 110°C. for one hour. The combined weight of 5 (or more) dried clots which have been obtained in this way can be de-

[2] Martin, C. J., *Proc. Roy. Soc. of New South Wales*, 1896, **30**, 150 (cited after Kellaway, *Med. J. of Australia*, 1929).

[3] Lamb, G., 1903 (cited after Kellaway).

*According to Mellanby (*J. Physiol.*, 1909, **38**, 441), the amount of tiger snake venom which coagulates 2 cc. of bird plasma in 4 minutes is .00003 mg.

termined fairly accurately in an ordinary quantitative balance. The amount of fibrin in 100 cc. of plasma is then obtained by multiplying by the proper factor (20, if the clots from 5 cc. of plasma are weighed). If a micro or assay balance is available, the clot from one cc. of plasma is ample for the determination.

The question could arise whether the substance precipitated by the venom is really fibrin, and if it is fibrin, whether the precipitation is complete. The simplest way to answer this question is by comparing the results obtained by this method with those obtained when the fibrin is precipitated by recalcifying the plasma. Accordingly, parallel micro-Kjeldahl determinations were made on fibrin clots from the same specimen of plasma by the 2 methods of precipitation. For example, in Table I are given the results for 2 cases which were

TABLE I.
Comparison of Results Obtained when Fibrin is Precipitated by Venom and by Calcium

Diagnosis		Estimation by Micro-Kjeldahl Method				Mg. Fibrin in 100 cc. Plasma Estimated by Weighing Directly Fibrin
		Mg. Fibrin N in 100 cc. Plasma		Mg. Fibrin in 100 cc. Plasma (N × 6.25)		
		Precipitated by Venom	Precipitated by Calcium	Precipitated by Venom	Precipitated by Calcium	Precipitated by Venom
Pneumonia	(1)	144.8	143.9			
	(2)	155.5	144.9			
	(3)		153.6			932.
	Mean	150.2	147.5	938.4	921.4	
Acute Gastro-enteritis	(1)	93.5	90.7			
	(2)	96.6	91.3			
	(3)	94.1	92.7			586.
	Mean	94.7	91.6	592.4	572.3	

thoroughly studied. It will be seen that the nitrogen values obtained by the 2 methods agree very closely. Furthermore, the fibrin values derived by multiplying the fibrin N by the factor 6.25 are almost identical with that found by directly weighing the dried clot obtained as described above. Of course the clot obtained by recalcifying the plasma cannot be weighed directly, since it contains calcium oxalate.

In addition, the results obtained by Wu's colorimetric method[4] were the same, whether the fibrin was precipitated by venom or by calcium. However, the colorimetric method itself is not entirely reliable, but this aspect will be taken up in a later paper.

[4] Wu, H., *J. Biol. Chem.*, 1922, **51**, 33.

Perhaps the principal advantage of this method of determining blood fibrin is its simplicity. The major difficulty is in obtaining the venom. However, since only a minute amount is required for each test, a small supply will last for a very long time. Even the quantities used in the present study (0.06 mg. of the dried venom per test) were far greater than necessary.

We wish to express our indebtedness to Dr. C. H. Kellaway, Director of the Walter and Eliza Hall Institute of Melbourne, Australia, who supplied us with the venom used in this investigation.

7863 P

Acquired Resistance of Liver Cells to the Toxic Action of Uranium Nitrate.*

WM. deB. MACNIDER.

From the Laboratory of Pharmacology, The University of North Carolina.

A statement has been made previously concerning the type, distribution and severity of the injury to the liver by the use through subcutaneous injection of a solution of uranium nitrate.[1, 2] In addition, observations were recorded[2] concerning the types of repair processes developing in the liver as a result of the reaction of the liver to this hepatoxic agent, and the resistance or lack of resistance which such processes manifested when the liver was subjected to the toxic action of chloroform, administered by inhalation.

The present series of experiments are concerned with the injury induced to hepatic epithelium by the subcutaneous use of uranium nitrate in the amount of either 2 or 4 mg. per kilogram, the type of repair process which is inaugurated by such injuries and the resistance which certain cells in such areas of repair may acquire to secondary intoxications by uranium. During the course of the experiments biopsy material has been obtained from the liver for cytological study and at such periods tests of hepatic function have been made by the use of phenoltetrachlorphthalein according to the technique devised by Rosenthal.[3]

*This investigation was made possible through a grant from The Josiah Macy, Jr., Foundation.

1 MacNider, Wm. deB., PROC. SOC. EXP. BIOL. AND MED., 1919, **16**, 82.

2 MacNider, Wm. deB., *Trans. Assn. Am. Physicians*, 1934, **49**, 14.

3 Rosenthal, S. M., *Bull. Johns Hopkins Hosp.*, 1922, **33**, 432.

Sixty-three dogs have been used in the experiments. Twenty-nine of the dogs were intoxicated with 2 mg. of uranium nitrate per kilogram. Ten of the animals failed to survive the intoxication. In the remaining 19 dogs there was an initial increase in the plasma concentration of phenoltetrachlorphthalein at the height of the intoxication which did not exceed 18%. In 8 of these animals the plasma was free from the dye in half an hour. In the remaining 11 dogs the removal of the dye was delayed. Biopsy material removed from the livers of such animals showed an increase in stainable lipoid material in the hepatic epithelium, and edema, but rarely vacuolation or necrosis of the cells. The vascular tissue of the liver failed to show any evidence of injury. An injury of this diffuse type and severity was followed by changes of repair which were usually complete within 9-20 days, and consisted in the formation of an essentially normal type of hepatic epithelium with a return of liver function to the normal as indicated by the use of phenoltetrachlorphthalein. When such animals were reintoxicated by the same amount of uranium nitrate there was no cytological evidence of epithelial resistance on the part of the liver and hepatic function as indicated by the use of the above mentioned dye as imperfectly as it indicates such function was depressed.

Thirty-four animals were intoxicated by the subcutaneous injection of 4 mg. of uranium nitrate per kilogram. Eighteen of these animals effected a survival and form the basis for the following observations. In this group the evidence of a disturbance in hepatic function was greater than that in the previous group. The initial plasma concentration of the dye was of a higher percentage, attaining a maximum of 24% in 2 of the dogs. The dye persisted in the plasma for as long as 2 hours. At such a stage of functional depression biopsy material removed from the livers showed an advanced edema, vacuolation and in areas necrosis of the epithelium. In less damaged cells lipoid material was present as large droplets or fused masses. Within 4 to 10 weeks in animals which have survived such a degree of liver injury there occurs an improvement in hepatic function, usually without its return to the normal. Biopsy material obtained from the liver at such a stage of recuperation has shown the repair process to the epithelium to have taken place through the formation of an atypical, flattened type of cell with proportionately large, deeply staining nuclei. The newly formed epithelium may be syncytial in structure. When such animals are reintoxicated with 4 mg. of uranium nitrate per kilogram it has been observed that the change in the type of cell in the liver develop-

ing as a process of repair has imparted resistance to the liver against this hepatoxic agent. There occurs but slight evidence of epithelial injury and hepatic function as indicated by the use of phenoltetrachlorphthalein may or may not be depressed from the pathological normal established by the liver as a result of an atypical type of fixed cell repair.

7864 C

Effects of a General Anesthetic (Sodium Amytal) on the Erythrocyte Count Following Hemorrhage.

ROBERT ELMAN, DAVID O. WEINER, AND WARREN H. COLE. (Introduced by Evarts A. Graham.)

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Although it is generally agreed that there is a reduction of the erythrocyte count after hemorrhage due presumably to a dilution of the circulating blood nearly all of the evidence of this phenomenon has been obtained some hours, *i. e.*, 24 or more, after bleeding. The behavior of the red blood count within a few hours after hemorrhage has not been extensively investigated, and among observers there is considerable disagreement, some finding an increase in the cell count indicating concentration and others finding a decrease indicating dilution.*

During other experiments it was noticed that the effect of general anesthesia seemed to have a very marked influence on the behavior of the red blood count after hemorrhage. A series of experiments were then undertaken, 13 in all. In 6 of them the blood was removed from the circulation from the femoral artery under local (novocaine) anesthesia, in the others after the induction of general anesthesia by means of the injection of sodium amytal intravenously. The amount of drug given did not exceed 50 mg. per kilogram of body weight in any case. However, many dogs received less than this amount for the injection was given slowly and discontinued as soon as the animal was deeply asleep. A single bleeding was done in each case amounting to from 3 to 4% of the body weight. Preliminary observations of the red blood cell count, venous, capillary and arterial, with the animal under general as well as without

*Lack of space precludes discussion of these observations.

anesthesia for a number of hours showed no significant alterations. In the 13 dogs observations were made before hemorrhage and for several hours after. The same position of the animal was maintained during the course of the experiment. Blood counts were obtained of capillary blood as well as venous, the latter being obtained through puncture of the right leg vein, the former from the base of the ear.

As can be seen from the accompanying charts there is a very definite difference between the 2 groups of experiments. In the dogs bled under general anesthesia there was in most cases not very marked changes in the red blood count. With a few exceptions, however, the change was an increase and in one instance there was a concentration as high as 31%. In contrast to these findings there was a reduction in the red blood count in each of the cases of hemorrhage performed without general anesthesia, indicating very defi-

CHART I.

Red Blood Counts (R.B.C.) before and after hemorrhage (local anesthesia). All counts are of capillary blood except those marked "V" which are venous.

Dog	Wt., Age, Sex and R.B.C. (in millions)	% of body Wt. bled	Red Blood Count After Bleeding (in millions) 1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.	Maximum change in R.B.C. %
1	21 kg. old male								
	6.52	3.8							—25
	6.36 V		5.5V	4.7V	died				died
2	5.4 kg. old male								
	5.4	3.7		5.0			3.9	3.9	—24
	4.92 V			5.3V			4.0V		—23
3	7.5 kg. young male								
	6.17	3.3			4.2	4.2			—32
	5.66 V				4.1V				—27
4	5 kg. young male								
	4.54	4.5			2.8				—35
	4.441 V				2.6V				—42
5	8 kg. old male								
	7.9	3.1	6.7	6.8		6.4	6.4		—19
	7.8 V		6.8V	6.9V	6.5V				—18
6	5 kg. young female								
	6.4	4.0	5.0		5.3		5.2		—20
	6.4 V				4.9V		5.0V		—29

nite dilution under these circumstances. This dilution amounted in one case to a reduction of 42% in the erythrocyte count, and in no case was less than 18%.

In a few instances red blood cell counts were repeated the next day, *i. e.*, 24 hours after the hemorrhage. During this time the animals had access to no food or water. In the dogs bled without general anesthesia there was in only one instance any further change in the red blood count, and it was an additional dilution.

In 3 instances in the other group, the 24-hour count was made after the effect of sodium amytal had worn off and when the dogs were conscious, alert and active. They showed a very marked re-

CHART II.

Red Blood Counts (R.B.C.) before and after hemorrhage (sodium amytal anesthesia). All counts are of capillary blood, except those marked "V," which are venous.

Dog	Wt., Age, Sex and R.B.C. (in millions)	% of body Wt. bled	Red Blood Count After Bleeding (in millions) 1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	Maximum change in R.B.C. %
7	5 kg. young female							
	5.88	3.0	5.4		5.5		6.0	— 7
	5.0 V		5.9V		5.7V		5.6V	+18
8	12 kg. young male							
	5.52	3.5	5.9			6.2	7.2	+31
	5.99 V		6.6V			7.6V	7.1V	+27
9	8 kg. young male							
	5.82	3.7					5.4	— 7
	5.53 V						6.0V	+ 8
10	7 kg. old female							
	5.72	3.7					5.6	— 1
	5.86 V						6.1	+ 5
11	13 kg. young male							
	6.4	3.5		5.7	5.8			—12
	5.9 V			6.6	6.1			+11
12	12.2 kg. old male							
	7.6	3.8	7.5	7.4	7.4		7.5	+ 2
	7.7 V		7.5V	7.6V			7.7V	— 2
13	10 kg. young male							
	7.4	4.1		7.3	7.9	7.5	7.3	+ 7
	7.3 V				8.3V	7.3V		+12

duction indicating very definite dilution. This dilution was in contrast to its absence while under the influence of the drug. Thus in dog 12 bled on January 11, 1935, there was practically no change (Chart II) during 9 hours. The next day at 8:00 a. m. he was awake and active. A blood count at this time revealed 6,730,000 red cells or a reduction of 11% from his normal level. By 2:00 p. m. of the same day it had gone down further to 5,570,000 cells or a reduction of 25%. During this time no fluid or food was ingested. Similarly, dog 9 was bled November 27, 1934, with a resulting slight dilution of capillary and concentration of venous blood (Chart II) during 8 hours. The next day recovery from the anesthesia was complete and the capillary count was 4,680,000, the venous 4,520,000, a dilution of 20 and 22% respectively. Dog 13, also under amytal, was bled on January 15, 1935, and revealed a change even sooner. Thus for 5 hours there was a slight concentration of the blood (Chart II). The animal awoke from the anesthesia shortly afterward, although he was kept in the same position for 2 more hours. During these 2 hours the capillary count dropped to 6,990,000, a reduction of 6%, and the venous count to 6,780,000, a reduction of 8%. The next day, however, the capillary count was 6,250,000 or a reduction of 16%.

Conclusions. There is an immediate dilution of capillary and venous blood in the dog after a single large hemorrhage performed under local anesthesia. This dilution is absent when a similar hemorrhage is performed after the induction of intravenous sodium amytal anesthesia. There may even be a concentration of the blood under this circumstance. As soon as the effects of the anesthesia wear off dilution promptly appears.

7865 P

Effect of Suprarenal Cortical Hormone on the Natural Resistance of Pituitarectomized Rats.

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We studied the effect of partial and complete pituitarectomy in rats on the natural resistance to histamine poisoning and correlated the anatomical changes in the suprarenal gland with the variations in resistance.[1] We noted that complete pituitarectomy in rats from 1 to 10 weeks after operation depressed the natural resistance of these animals to histamine poisoning. The M.L.D. was one-fifth to one-third that for normal rats. This decrease in resistance, we found, was associated with involutional changes of the suprarenal gland, such as hemorrhage into or atrophy of the inner zones of the cortex.[2] Rats in which the posterior lobe and a large portion of the anterior lobe were removed showed a similar drop in resistance and atrophic changes in the suprarenal cortex occurred, but where a large fragment of the anterior lobe remained no depression in resistance to histamine occurred and the suprarenal glands were normal. We concluded that the drop in natural resistance following pituitarectomy in the rat is probably secondary to the atrophic changes of the suprarenal cortex induced by the withdrawal of the adrenotropic hormone of the anterior lobe.*

In the present studies the effect of the repeated injections of suprarenal cortical hormone, in pituitarectomized rats, on the natural resistance of these animals to histamine poisoning was observed.

Eighteen totally pituitarectomized adult albino rats were divided into 2 groups. Ten of these were given, during a period of 6 days, 2 injections daily of cortical hormone in amounts of 1 cc. per rat per day [equivalent to 40 gm. of fresh ox cortex (eschatin, Parke, Davis and Co.)] The interval between pituitarectomy and the day the treatments were begun varied from 5 to 8 days, but in one instance the rat had been pituitarectomized 89 days prior to the injections of the cortical hormone. Eight pituitarectomized rats received no cortical hormone injections. Those rats treated with

1 Perla, D., and Rosen, S. H., in press.

2 Perla, D., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 655.

* The literature on the evidence of the interrelationship of the anterior lobe of the pituitary and the suprarenal cortex is reviewed in an earlier paper[2] and by Shumacker and Firor.[3]

3 Shumacker, H. B., and Firor, W. M., *Endocrin.*, 1934, **18**, 676.

TABLE I.

Effect of Repeated Injections of Suprarenal Cortical Hormone on Natural Resistance of Pituitarectomized Adult Albino Rats to Histamine Poisoning.*

Rat No.	Sex	Operat. Proced.	Days bet. Opera. and Injec. of Cortin	Days bet. Opera. and Injec. of Histam.	Wt. at Opera. gm.	Wt. begin. of Cortical treatment gm.	Wt. time of Histamine injec. gm.	Amt. Hist. in mg. per Kg. body wt.	Result	Comment
					Rats Treated with Cortin					
1	M	C.P.	7	14	205	183	196	700	Survived	
2	F	”	6	14	191	178	180	800	”	
3	M	”	5	13	271	221	233	800	Died	
4	M	”	5	13	144	140	153	700	Survived	
5	M	”	8	14	165	140	145	700	Died	Survived 2 da. after hist. inj.
6	M	”	8	14	174	150	161	800	Survived	
7	F	”	7	12	154	125	132	800	”	
8	M	”	6	11	157	138	130	700	Died	Mkd. atrophy of supraren.
9	M	”	5	11	149	140	152	600	Survived	
10	M	”	89	95	167	145	154	800	Died	Extreme atrophy supraren.
					Rats Not Treated with Cortin					
11	F	”	No Cortin	12	165		150	400	Died	
12	F	”	”	13	152		117	400	Survived	
13	M	”	”	54	205		170	700	Died	
14	F	”	”	50	191		170	600	”	
15	M	”	”	11	179		160	200	Survived	
16	F	”	”	12	152		124	200	Died	
17	F	”	”	15	161		140	300	”	
18	M	”	”	16	180		158	400	”	
19	M	O.C.	”	14	210		192	900	Survived	
20	F	”	”	12	212		196	600	”	
21	F	Normal	”				165	900	”	
22	M	”	”				185	900	Died	
23	F	”					160	1000	”	
24	M	”					190	1000	”	

*All treated rats received 0.5 cc. of cortical hormone (equivalent to 40 gm. of whole cortex) twice daily over a period of 6 days prior to the injection of histamine.

Key to Chart: C.P. = Complete pituitarectomy. O.C. = Pituitary exposed but not removed.

cortical hormone were injected 6 days after treatment was begun with histamine in amounts ranging from 600 to 800 mg. of histamine per kg. of body weight (ergamine acid phosphate). Of 4 normal rats, 2 received 900 mg. of histamine per kg. and 2 received 100 mg. per kg. Two operated control rats received 600 and 900 mg. of histamine per kg. of body weight. The untreated pituitarectomized rats received histamine in amounts ranging from 200 to 700 mg. per kg. of body weight.

Results. The rats treated with cortical hormone appeared in most instances to be clinically improved and gained in weight, though no effect anatomically was observed on the degree of atrophy of the suprarenal cortex.†

Of the 10 pituitarectomized rats treated with cortical hormone the one receiving 600 mg. of histamine per kg. of body weight survived. Of 4 receiving 700 mg. per kg., 2 survived. Of 5 receiving 800 mg. per kg., 3 survived.

Of the 8 pituitarectomized rats not treated with cortical hormone and injected with histamine, of 2 rats receiving 200 mg. of histamine per kg. of body weight, one died; one receiving 300 mg. per kg., died; of 3 receiving 400 mg. per kg., 2 died; one receiving 600 mg. per kg. and one receiving 700 mg. per kg. died. The 2 operated controls (receiving no cortical hormone) survived 600 mg. and 900 mg. of histamine per kg. Of the normal controls the 2 receiving 1,000 mg. of histamine per kg. died; and of 2 receiving 900 mg. per kg., one died.

The repeated injections of large amounts of suprarenal cortical hormone raised the natural resistance of totally pituitarectomized adult rats to histamine poisoning. In some instances the resistance was raised almost to the level of normal rats. This is particularly marked during the first 2 weeks after operation, the period during which the most severe depression in natural resistance occurs. The M.L.D. of histamine for pituitarectomized rats within 2 weeks after operation varies from 200 to 300 mg. per kg. of body weight.[1] Pituitarectomized rats treated with cortical hormone for 6 days prior to the injection of histamine survived, in many instances, 800 mg. of histamine per kg. of body weight.

† Shumacker and Firor[3] mention that cortical hormone does not modify the anatomical changes of the suprarenal cortex induced by pituitarectomy. Kalk[4] treated a patient suffering with pituitary cachexia, with suprarenal cortical hormone and noted prompt improvement of the patient's condition. He believed that the pituitary cachexia is a result of the loss of suprarenal cortical function.

[4] Kalk, H., *Dtsch. Med. Wchnschr.*, 1934, **1**, 893.

These rats gained in weight in most instances and were apparently in better physical condition than untreated rats.

The effectiveness of the parenteral administration of any given amount of cortical hormone probably varies to some degree with the total cortical hormone which may be still available in the involuted suprarenal cortex.‡

These observations further support the hypothesis that the depression in natural resistance following pituitarectomy is due to the impairment of the function of the suprarenal cortex resulting from the withdrawal of the adrenotropic hormone of the anterior lobe.

7866 C

The Age Factor in Responsiveness to Gonadotropic Hormones.

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We reported[1, 2] that in very young rats (6-12 days old) A.P.L. (anterior pituitary-like hormone of pregnancy urine) leads only to the luteinization of theca cells but not to the formation of corpora lutea from granulosa cells. This theca-reaction is accompanied by continuous vaginal oestrus. This type of reaction to A.P.L is very similar to that observed in hypophysectomized rats[3] and therefore the possibility of its being due to insufficient pituitary function at this early age had to be considered.

Corey[4] and Swezy and Evans[5] stated that pituitary implants have no effect on the ovaries of the rat during embryonic development, and during the first days of life. In order to see whether the effect of A.P.L. is different from that of the pituitary gonadotropic hormone during the first days of life, we repeated our earlier A.P.L.

‡ It is of interest that in a pituitarectomized rat in which treatment was begun 95 days after operation, the rat gained in weight, but did not survive 800 mg. of histamine per kg. of body weight. Its suprarenals were found to be reduced to the size of a pinhead.

1 Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 647.

2 Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 780.

3 Collip, J. B., Selye, H., and Thomson, D. L., *Nature*, 1933, **131**, 56.

4 Corey, E. L., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 498.

5 Swezy, O., and Evans, H. M., *Anat. Rec.*, 1931, **50**, 189.

experiments with a potent sheep pituitary extract. This extract was prepared as follows:

Sheep glands which had been partly defatted and dehydrated with acetone were extracted twice with 4 volumes of 70% acetone containing 1% ammonia. The residue was extracted with 1% aqueous ammonia, $Ca_3(PO_4)_2$ being used as an aid to filtration. The filtrate was saturated with $(NH_4)_2SO_4$ and the resultant precipitate was dissolved in water. Two volumes of acetone were added to this solution and the precipitate which formed was collected and dissolved in water, so that 1 cc. of the solution represented ¼ gm. of the original glands. Immature rats injected subcutaneously twice daily with ½ to 1 cc. of this extract manifested vaginal oestrus in 72 to 96 hours and showed enlarged luteinized ovaries (40 to 60 mg.) on the fifth day. A standard powder was also prepared from the above mentioned acetone precipitate.

Eleven female rats were injected with ⅛ to 1 cc. of this extract twice daily over a period of 8 to 44 days.. They were killed 24 hours after the last injection. The treatment did not induce oestrus and the ovaries of 2 animals killed on the 8th day, and of 2 killed on the 11th day, showed no detectable changes, except that in the 2 latter cases a few hemorrhages into the theca were seen. Thecal luteinization did not occur. The first vaginal oestrus was observed on the 14th day of treatment—that is, at a time when the animals were 26 days of age. Thenceforward the ovaries reacted with formation of corpora lutea of granulosa origin, and irregular cycles appeared. The maximum ovarian weight increase was observed around the 25th day of treatment (120 mg.); thereafter the ovaries involuted in spite of the continued treatment, the animals having lost their sensitivity to this preparation. It appears from these experiments that in the very young rat the gonadotropic preparations from pregnancy urine do not act in the same way as those obtained from the pituitary. While the former lead to theca-luteinization and continuous vaginal oestrus, the latter have no effect on the ovary and do not cause oestrus.

In another experiment the ovaries of six 12-day-old rats were implanted into the spleens of 6 females which were ovariectomized 2 weeks before the transplantation was performed. Even though the transplant "took" in all these cases, no follicle maturation, corpus luteum formation or theca luteinization was observed, and the hosts showed no vaginal oestrus. This experiment corroborates the view that the rat ovary is insensitive to the pituitary sex hormone during the first days of life.

The development of the Graafian follicle and its capacity to undergo transformation into a corpus luteum may be regarded as divisible into 3 different periods. The first stages of development are found in the ovary of rats between the first and the eighteenth day of life. At this period the granulosa cells do not respond to any known gonadotropic hormone, and the only effect that one can see after the administration of A.P.L. is the transformation of theca cells into theca-lutein cells,[1, 2] while anterior pituitary sex hormone does not seem to have any effect at all. The irresponsiveness of such immature ovaries to gonadotropic hormones is in the ovary itself and not in its hormonal environment, because these ovaries will not form mature follicles and corpora lutea even if they are transplanted into adult female castrates.

The second stages of development are found in the ovaries of rats after the 18th day of life, and in the ovaries of hypophysectomized animals. Up to this stage of maturation no pituitary hormones are needed. The experiments of Swezy[6] have shown that ovogenesis is not impaired after hypophysectomy, and since we have frequently found mitotic figures in the granulosa of hypophysectomized rats we feel that the maturation of the granulosa must also be independent of the pituitary hormones up to a certain stage in the development of the follicle. In this second stage, the follicle is able to respond to the pituitary follicle-stimulating hormone, but not to A.P.L. If A.P.L. is administered to hypophysectomized animals, only thecal luteinization will take place. If it is administered to the immature animal (around the 18th day of age) the follicles of which are also in this stage of development, the same preparation will produce follicle maturation with the subsequent luteinization of the granulosa only because the experimental animal's own pituitary is able to participate in the stimulation of the ovary,[7, 8]

The third stages of development are found in the ovaries of animals in full oestrus or of hypophysectomized animals after administration of follicle-stimulating hormone as obtained from castrate urine or pituitary tissue. This is the stage of full follicular development. At this time, and only at this time, A.P.L. has a direct effect on the granulosa cells, which it is able to transform into corpus luteum cells.

This explains why A.P.L. may lead to the formation of a true

[6] Swezy, O., *Ovogenesis and Its Relation to the Hypophysis,* Science Press, Lancaster, Pa., 1933.

[7] Selye, H., Collip, J. B., and Thomson, D. L., *Endocrinol.*, 1933, **17**, 404.

[8] Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 262.

corpus luteum if injected into a rabbit immediately after hypophysectomy, since in this species fully mature follicles are almost continuously present in the ovary. It further explains why the injection of A.P.L. into guinea pigs does not lead to the formation of corpora lutea except in the presence of a large follicle. Since this species responds to A.P.L. in a manner similar to the hypophysectomized rat or rabbit, one may assume that the hypophysis of the guinea pig does not participate with the injected A.P.L. in follicular stimulation. This interpretation would also make Engle's[9] experiments on monkeys more comprehensible. The monkey seems to react very much like the guinea pig, in that here again A.P.L. produces luteinization only after the follicle has responded to hypophyseal stimulation.

It seems necessary at the present time to postulate 2 hypophyseal gonadotropic hormones, one follicle-stimulating and one that luteinizes the theca and the mature granulosa while it has no effect on the immature granulosa cells. The so-called "Prolan A" of menopausal urine appears to consist chiefly of the former, or at least to resemble it closely, whereas the placental hormone "A.P.L." of pregnancy urine ("Prolan A plus Prolan B" of Zondek's original terminology) is more comparable, in its biological relations, to the luteinizing fraction.

7867 C

Effect of Iodine and Desiccated Thyroid on Anterior Pituitary of Goitrous and Thyroidectomized Rabbits.*

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It has been known for nearly a century[1] that individuals and animals with endemic goiter or cretinism have hypertrophy of the anterior pituitary. In man pituitary weights up to 3 gm. have been recorded, while in rabbits of approximately 2 kg. body weight with large parenchymatous goiters we have observed pituitary weights of 0.070 gm., whereas our normal average weight of the pituitary of rabbits of this size is 0.020-0.022 gm. Rogowitsch[2] first demon-

9 Engle, E. T., *Endocrinol.*, 1934, **18**, 513.

*Aided by a grant from the Ella Sachs Plotz Foundation.

1 Nièpce, B., *Traité du goître et du crétinisme*, Paris, 1851.

2 Rogowitsch, N., *Ziegler's Beitrag.*, 1889, **4**, 453.

Rabbit No. and Sex	Wt. at Autopsy gm.	KI or Des. Thyroid No. and Size Doses mg.	Duration Exper. days	Thyroidec. Dura. Life After days	Wt. Pituitary mg.	Histology Pituitary*	Wt. Thyroid mg.	Histology Thyroid
				TABLE 1 A				
1312-F	2910	12—1.25	42	42	52	Mod. hypertrophy Acid. greatly reduced	None found	
1331-F	2287	12—1.25	43	43	45	Mod. hypertrophy Acid. greatly reduced	None found	
1327-M	2128	12—1.25	43	43	61	Mod. hypertrophy Acid. reduced	None found	
1352-F	2978	12—1.25	42	42	39	Mod. hypertrophy Acid. reduced	Small fragment	Colloid early
				TABLE 1 B				
1431-M	1363	24—150	25	220	19	Not hypertrophied Acid. normal	None found	
1436-M	1058	38—150	39	244	22	Not hypertrophied Acid. abundant	None found	
1441-M	1477	39—150	40	221	23	Not hypertrophied Acid. abundant Pars interm. active	Small fragment	Colloid
1442-F	1688	38—150	39	220	25	Not hypertrophied Acid. abundant Pars interm. active	Small fragment	Colloid
1447-F	1723	39—150	40	221	18	Not hypertrophied Acid. abundant Pars interm. active	Large fragment	Colloid

TABLE 1 C

1297-M	2095	1—2.5	3	—	39	Mod. hypertrophy Acid. greatly reduced	657	Colloid early
1298-F	1965	2—2.5	6	—	30	Mod. hypertrophy Acid. possibly reduced	424	Nearly colloid
1299-M	1799	3—2.5	9	—	19	Very sl. hypertrophy Acid. abundant	252	Colloid
1329-F	1771	4—2.5	12	—	22	Very sl. hypertrophy Acid. abundant	343	Colloid
1332-M	1535	5—2.5	15	—	15	Not hypertrophied Acid. abundant	398	Colloid
1325-F	1556	6—2.5	18	—	19	Not hypertrophied Acid. abundant	354	Colloid

TABLE 1 D

1470-M	2221	48—100	49	—	18	Not hypertrophied Acid. normal	236	Colloid
1471-M	2140	48—100	49	—	18	Not hypertrophied Acid. normal	219	Colloid
1473-M	1960	48—100	49	—	17	Not hypertrophied Acid. normal	152	Colloid
1459-M	2022	48—100	49	—	18	Not hypertrophied Acid. normal	169	Colloid

*Sections stained with (1) H & E and (2) Mallory's Connective Tissue.

strated that the anterior pituitary cells of rabbits underwent hypertrophy following thyroidectomy, an observation which has been confirmed by most subsequent observers for many species of mammals including man, as well as lower orders (amphibians, reptiles). Pituitary hypertrophy following thyroidectomy is most pronounced in young rabbits and may be overlooked in old adults.

During the past 4 years we have readily produced large parenchymatous goiters in prepuberal rabbits by maintaining them on a diet of alfalfa hay of low iodine content, whole oats, tap water and the daily injection of methyl cyanide up to 0.1 cc. in 20% solution intramuscularly. This material has made it possible to compare in the same breeds of rabbits under similar environmental conditions the hypertrophic changes in the anterior pituitary associated with parenchymatous goiter with similar changes following thyroidectomy.

As Rogowitsch has pointed out, all the cells of the anterior pituitary undergo hypertrophy following thyroidectomy and coincident with this there is a progressive relative increase in the chromophobic and a decrease in the stainable acidophilic cells, which in the rabbit attains its maximum between the 30th and 60th day. Contrary to the opinion expressed by Bryant,[3] we believe that the apparent increase in chromophobic cells is due to a degranulation of acidophilic cells.

Histologically the changes in the anterior pituitary in parenchymatous goiter and following thyroidectomy are practically identical and comprise 2 major alterations: (1) an increase in the size of all the glandular cells and (2) a decrease of the stainable acidophilic granules which may progress to complete disappearance.

Effect of iodine on the hypertrophic anterior pituitary of thyroidectomized rabbits. We have given iodine in doses of 1.25 to 2.5 mg. KI intraperitoneally 2 and 3 times weekly for one month or more, both beginning at the time of thyroidectomy and beginning approximately one month after thyroidectomy. Iodine in these large amounts does not arrest or prevent the hypertrophy of the anterior pituitary, nor does it arrest or prevent the loss of acidophilic granules, provided the thyroidectomy is complete. Iodine, when administered after hypertrophy of the pituitary has occurred, does not restore the acidophilic granules nor does it bring about a shrinkage in the size of the hypertrophic cells or reduce the weight of the pituitary. (Table 1 A.)

Effect of desiccated thyroid on the hypertrophic anterior pituitary of thyroidectomized rabbits. We have given desiccated thyroid in

[3] Bryant, A. R., *Anat. Rec.*, 1930, **47**, 131.

doses of 0.1 gm. 3 times weekly and up to 0.2 gm. daily beginning both at the time of thyroidectomy and also after a month or more following thyroidectomy. Desiccated thyroid in these doses completely prevents the hypertrophy of the anterior pituitary following thyroidectomy and also prevents the loss of acidophilic granules. If desiccated thyroid is given after the hypertrophy of the anterior pituitary caused by thyroidectomy has occurred, it brings about a restoration of the acidophilic secretion granules and causes a decrease in the size of the anterior pituitary cells individually and in the weight of the gland as a whole. (Table 1 B.)

Effect of iodine on the hypertrophy of the anterior pituitary of

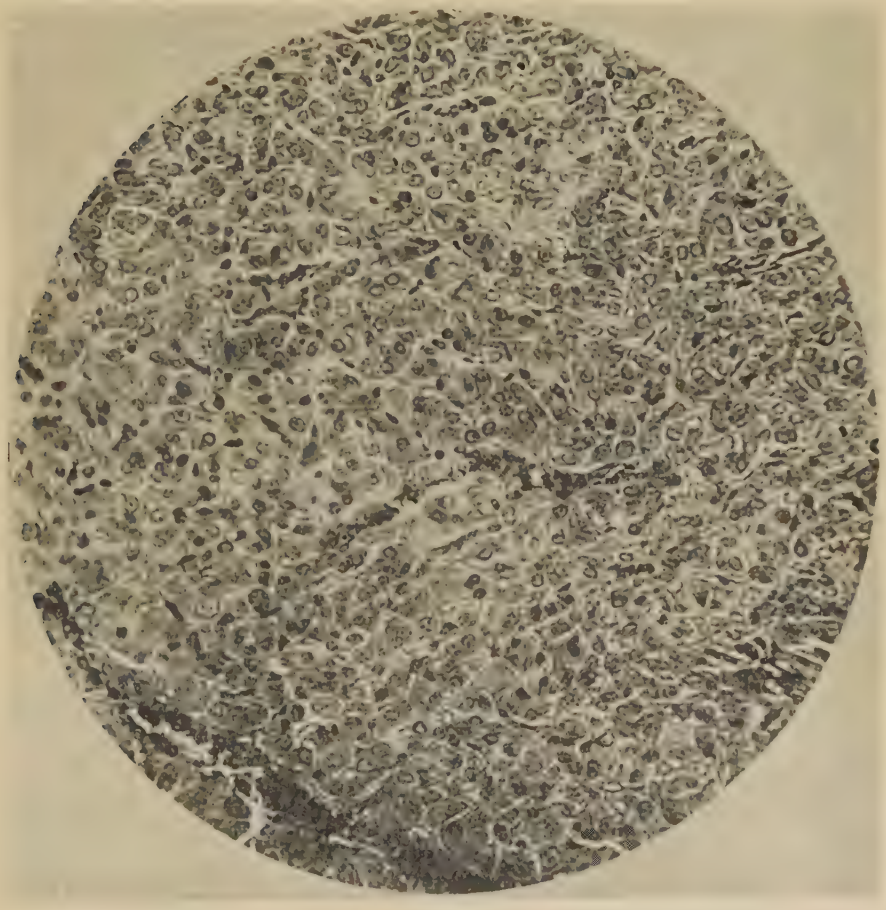

FIG. 1. Rabbit 1297. H and E stain. X 240. 1 injection 2.5 mg. KI, 3 days. Acidophilic granulations beginning to appear, cells large, only few pyknotic nuclei.

parenchymatous goiter. Iodine given in doses of 2.5 mg. KI 2-3 times weekly causes a rapid restoration of the acidophilic secretion granules and a shrinkage in the size of the cells. As determined by the comparative weights of the pituitaries there is also a great decrease in size. These changes in the anterior pituitary are obvious as early as the third day following the first dose of 2.5 mg. KI and by the ninth day the acidophilic granules are practically restored to normal staining intensity. The involution of the cells (shrinkage in volume and weight of gland) is far advanced but may not be complete in this time period. (Table 1 C.)

Effect of desiccated thyroid on the hypertrophy of anterior pituitary of parenchymatous goiter. Desiccated thyroid in doses of 0.1

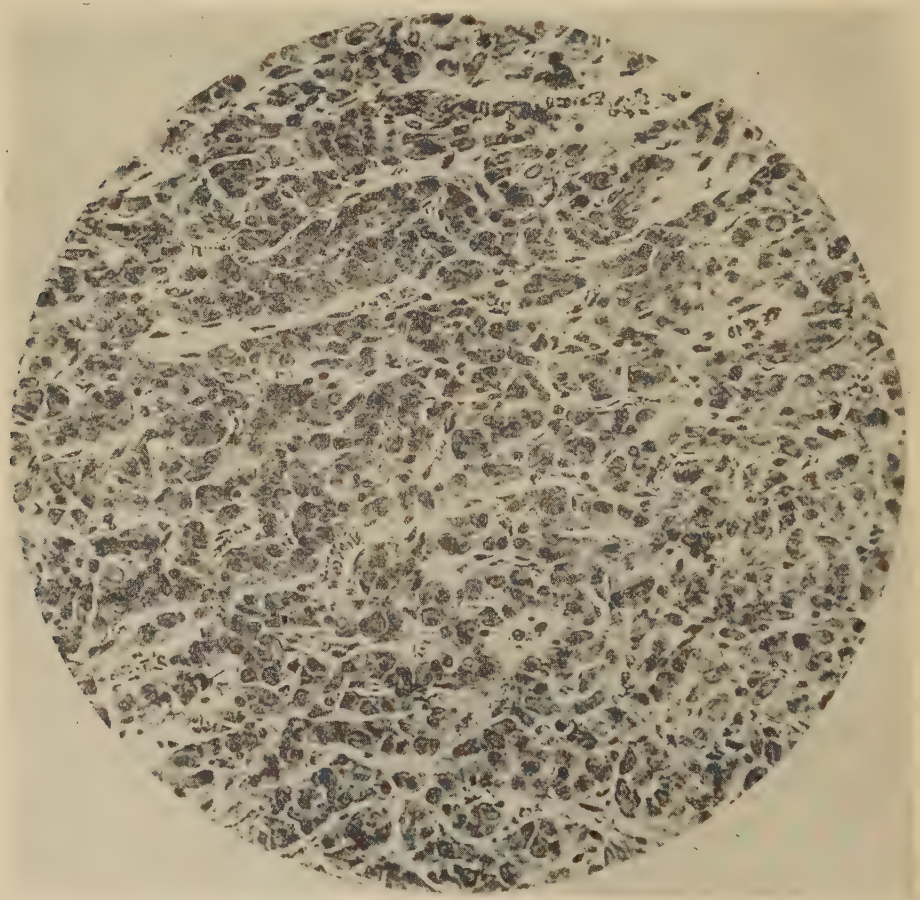

FIG. 2. Rabbit 1298. H and E stain. X 240. 2 injections 2.5 mg. KI, 6 days. Acidophilic granules more numerous. Cells and nuclei are smaller. More pyknotic nuclei.

gm. daily produces changes similar to those caused by iodine and also similar to those which desiccated thyroid in the same doses produces in the hypertrophic anterior pituitary following thyroidectomy. (Table 1 D.) Severinghaus, Smelser and Clark[4] observed that the acidophiles were larger and stained more brilliantly in thyroid fed rats.

It is evident, therefore, that desiccated thyroid will prevent the anterior pituitary changes and if present will restore the gland to the anatomical condition approximating normal, whether the thyroid

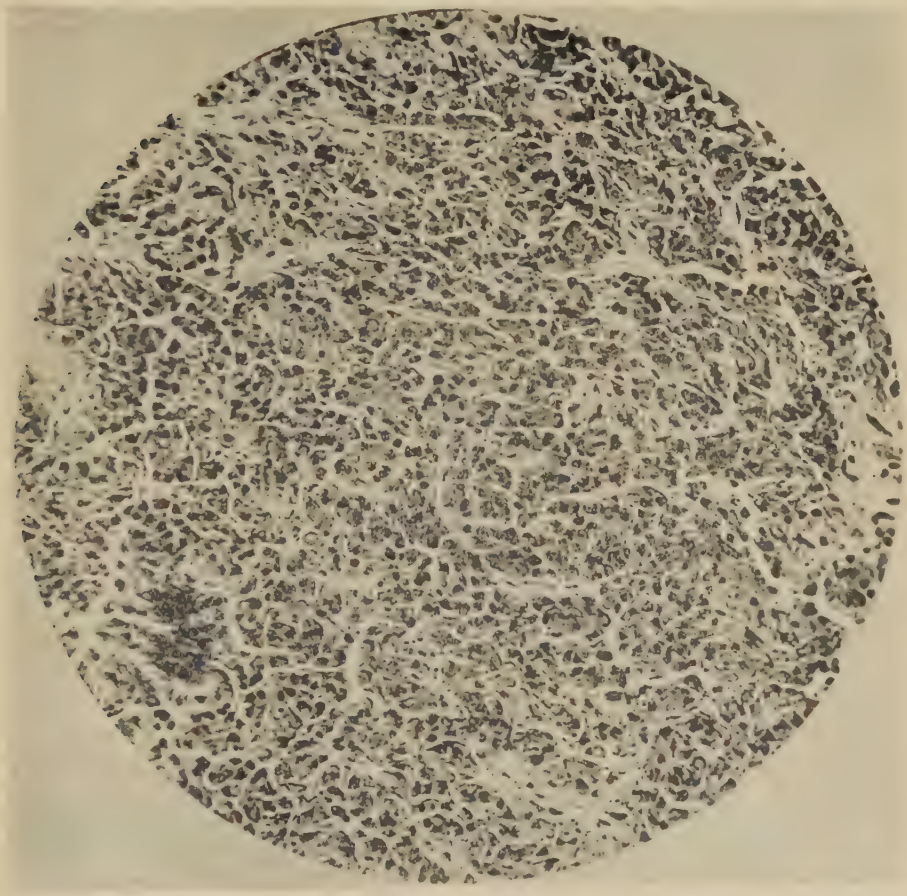

FIG. 3. Rabbit 1299. H and E stain. X 240. 3 injections 2.5 mg. KI, 9 days. Acidophilic granules abundant. Striking decrease in size of cells and condensation of nuclei.

4 Severinghaus, A. E., Smelser, G. K., and Clark, H. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1125.

is removed or intact. On the other hand iodine will restore the hypertrophic pituitary of parenchymatous goiter to normal as quickly and as effectively as desiccated thyroid, but it has no detectable effect on the pituitary after thyroidectomy. Evans and Simpson[5] have found that fresh beef thyroid fed to normal rats for 5 weeks caused an appreciable decrease in the weight of the pituitary and Loeser[6] showed that the pituitaries of iodine fed normal rats contained more thyrotropic substance than those of controls.

These facts suggest that only thyroxine is capable of preventing the hypertrophy of the pituitary after thyroidectomy and in parenchymatous goiter, and of restoring such glands to normal, and that iodine is effective only when the animal is capable of utilizing it in the elaboration of thyroxine. The thyroid secretion affects the anterior pituitary as strikingly as the anterior pituitary hormone affects the thyroid. From these results it would appear certain that the acidophilic granules are true secretion granules, decreasing with increased functional activity of the cells and increasing with physiologically decreased functional activity. This evidence could be further interpreted as indicating that the acidophilic granules contain the thyrotropic factor, although direct tests by several investigators have failed to demonstrate that there is a significant decrease in thyrotropic potency of the pituitary after thyroidectomy.

7868 P

Anti-Anemia Potency of Liver After Gastrectomy in Swine.*

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(Introduced by H. G. Barbour.)

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The introduction by Minot and Murphy[1] of liver therapy for pernicious anemia, and the subsequent discovery by Sturgis and Isaacs[2] of the efficacy of desiccated stomach have stimulated interest in the interrelationship between liver and stomach in the etiology

[5] Evans, H. M., and Simpson, M. E., *Anat. Rec.*, 1930, **45**, 215.

[6] Loeser, A., *Klin. Wchschr.*, 1934, **13**, 533.

*Aided in large part by financial grants from the Committee on Scientific Research of the American Medical Association.

of the anemia and in the production of the anti-anemia principle. Castle's demonstration[3, 4] of an "intrinsic factor" present in normal gastric juice but absent from the stomach of the patient with pernicious anemia, and his experiments[5, 6] showing the interaction of this factor with an unknown food factor to form a product that is effective in the treatment of the disease crystallized the view that the liver serves chiefly to store and elaborate the substance prepared by the stomach. The many lines of collateral evidence converging on this conclusion were recently reviewed by Klumpp and Koletsky.[7] However, no direct, irrefutable experiment had yet been reported proving whether or not in the absence of the stomach the liver can elaborate the anti-anemia principle effective in the therapy of pernicious anemia. We undertook to answer this question and a number of its important corollaries.

An important step in the program involved the estimation of the anti-anemia potency of livers obtained from totally gastrectomized animals. It is desired here to report briefly a few preliminary observations.

Seven healthy young swine were successfully gastrectomized. The animals were fed an adequate diet lacking only in the main sources of the anti-anemia principle. Growth and development apparently proceeded normally. The complete blood pictures were closely followed and, with one possible exception, no striking changes were observed. Animals were sacrificed at intervals between the second and sixth month after gastrectomy. The spinal cord and bone marrow appeared normal on histopathological examination. The livers were extracted by the method used in making Lilly's Solution Liver Extract No. 343. The adequacy of our extraction technic was proved by clinical comparison of commercial preparations with our control extracts made from normal liver. The assay of the experimental extracts is being performed on severe, untreated cases of classical pernicious anemia, following the method recently described by Dameshek and Castle.[8]

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†National Research Council Fellow in Medicine, 1934.

1 Minot, George R., and Murphy, Wm. P., *J. Am. Med. Assn.*, 1926, **87**, 470

2 Sturgis, Cyrus C., and Isaacs, Raphael, *J. Am. Med. Assn.*, 1929, **93**, 747.

3 Castle, Wm. B., Townsend, W. C., and Heath, C. W., *Am. J. Med. Sci.*, 1930, **180**, 305.

4 Castle, Wm. B., Heath, C. W., and Strauss, M. B., *Am. J. Med. Sci.*, 1931, **182**, 741.

5 Castle, Wm. B., *Am. J. Med. Sci.*, 1929, **178**, 748.

6 Castle, Wm. B., and Townsend, W. C., *Ibid.*, 764.

7 Klumpp, T. G., and Koletsky, S., *Ann. Int. Med.*, 1935, in press.

8 Dameshek, Wm., and Castle, Wm. B., *J. Am. Med. Assn.*, 1934, **103**, 802.

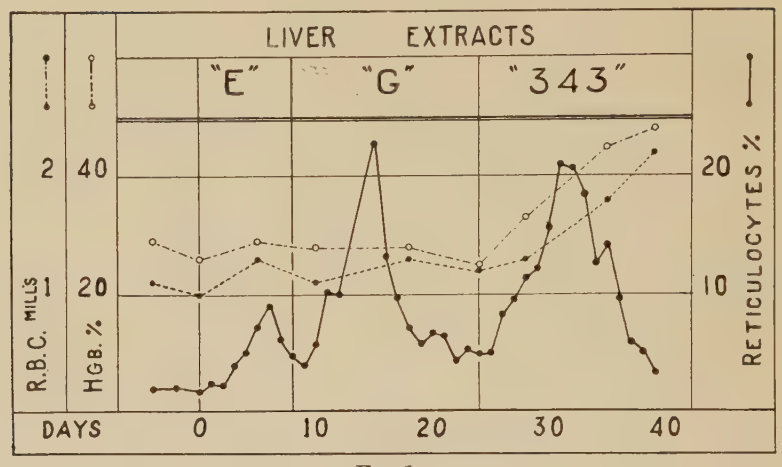

FIG. 1.

A typical result is shown in the accompanying chart which depicts the hematological response of a patient with pernicious anemia to the administration of liver extracts obtained from 2 pigs, "G" and "E", sacrificed at 2 and 4 months, respectively, following gastrectomy. In this particular experiment Lilly's Solution Liver Extract No. 343 served as control. During the period of assay the patient received daily intramuscular injections of 2 cc. of extract, each cc. being derived from 5 gm. of original liver. It is clearly evident that extract "G" is less potent than normal pig liver, and that extract "E" is considerably weaker than "G". In general, our results confirm and amplify those recently reported by Bence.[9]

Summary. The results show definitely that after total gastrectomy in the pig the anti-anemia potency of the liver becomes progressively depleted, and that a significant loss is already detectable as early as the third month.

[9] Bence, J., *Z. für klin. Med.*, 1933, **126**, 127.

7869 P

Influence of Vitamin C on Development of Skin Sensitivity to Neoarsphenamine in the Guinea Pig.

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Scorbutic guinea pigs have a reduced skin reactivity.[1, 2, 3] Frei[4] sensitized guinea pigs to neoarsphenamine. Mayer and Sulzberger[5] reported that diet exerted an important influence in neoarsphenamine sensitization in guinea pigs. The present experiment demonstrates that guinea pigs on a scorbutic diet fail to develop as definite skin sensitivity to intradermal injections of neoarsphenamine as animals on a diet sufficient in Vitamin C.

Guinea pigs in groups B, C, D and E (Table I) were fed a grain mixture and carrots. Group B received in addition at various intervals 2 mg. ascorbic acid by mouth. Animals of Group A received the grain mixture, kale, hay and some carrots. These animals of group A did not develop the cardinal symptoms of scurvy but were maintained in a subacute state. They did not increase in weight during the experiment as did the animals in the other groups. The animals were fed these diets for 15 days before receiving the first intradermal injection.

For the sensitizing dose, groups A, B and C received intradermally 0.1 cc. of a 0.15% solution of neoarsphenamine in 5% C.P. dextrose, the solution being made from freshly boiled, glass-redistilled water. Control groups, D and E, received intradermally 0.1 cc. of the same solution without the neoarsphenamine. The provocative dose for groups A, B and E was the same as the sensitizing dose. For groups C and D the sensitizing and provocative doses were reversed (Table I). There was an interval of 40 days between the 2 doses.

Results. In group A (subacute scurvy), the *sensitizing* dose gave a smaller reaction than it did in group B. There was little or no difference in the reactions produced by the sensitizing and provoca-

[1] Prausnitz, C., and Schilf, F., *Deutsch. Med. Wchnschr.*, 1924, **50**, 102.

[2] Bieling, R., *Deutsch. Med. Wchnschr.*, 1927, **53**, 182, 228.

[3] Arkwright, J. A., and Zilva, S. S., *J. Path. and Bact.*, 1923, **27**, 346.

[4] Frei, W., *Klin. Wchnschr.*, 1928, **22**, 1026.

[5] Mayer, R. L., and Sulzberger, M. B., *Arch. f. Dermat. u. Syph.*, 1931, **163**, 745.

TABLE I.

Group.	No.	Diet	Sensitizing Dose. Left Flank. *				Provocative Dose. Right Flank.				
				Days after injection				Days after injection			
			Soln. used	1st	3rd	8th	Soln. used	1st	3rd	4th	6th
A	201	Scorbutic	Neoarsphenamine + Dextrose	S	O	O	Neoarsphenamine + Dextrose	S	O	O	O
	209			S	O	O		O	S	S	O
	210			S	S	O		S	S	O	S
	211			S	S	O		S	S	O	O
	212			S	S	O		O	O	O	O
B	214	Anti-Scorbutic	Neoarsphenamine + Dextrose	D	S	O	Neoarsphenamine + Dextrose	M	D	O	O
	200			D	O	O		D	O	O	O
	202			D	D	O					
	204			D	D	O		M	S	O	O
	213			S	S	O		M	D	M	O
								M	M	M	D
C	215	Anti-scorbutic	Neoarsphenamine + Dextrose	S	D	O	Dextrose	S	O	O	O
	219			S	S	O		O	O	O	O
	221			S	S	O		O	O	O	O
	240			S	S	O		O	O	O	O
D	206	Anti-scorbutic	Dextrose	S	O	O	Neoarsphenamine + Dextrose	S	S	M	M
	208			S	O	O		S	O	O	O
	220			S	O	O		S	O	O	O
E	203	Anti-scorbutic	Dextrose	S	O	O	Dextrose	O	O	O	O
	205			S	O	O		S	O	O	O
	207			S	O	O		O	O	O	O

* Interval of 40 days between sensitizing and provocative dose.

M = Marked reaction; a very marked redness covering an area not less than the size of a large bean.

D = Definite reaction; a definitely red area about the size of a pea.

S = Slight reaction; a small faintly red area.

O = No reaction.

tive doses in group A. This shows that skin sensitivity is not developed in animals suffering from subacute scurvy. The provocative dose in group B resulted in very marked skin reactions indicating that these animals were sensitized. Groups C, D and E serve as controls. They show that no reaction is produced by dextrose, group E; that guinea pigs receiving neoarsphenamine and then dextrose for the provocative dose do not react, group C; and that animals receiving dextrose in the first injection are not sensi-

tive to neoarsphenamine, group D. Pig No. 206 gave a "spontaneous flare-up", as described by Sulzberger,[6] after the first injection of neoarsphenamine, due to *developing* skin sensitivity. Group B, which received some ascorbic acid in addition to carrots, grew at the same rate as groups C, D and E which did not receive ascorbic acid, but developed more marked skin reactions to the sensitizing dose than groups A and C.

This experiment carried out during October-December, 1934, confirms the results obtained in a previous experiment on definitely scorbutic pigs during June-August, 1934.

[6] Sulzberger. M. B., *Arch. Derm. and Syph.*, 1929, **20**, 669.